REMARKS

Claims 1 to 56 are pending in this application and are rejected. Applicants are herein amending claims 33 and 56. Applicants request reconsideration of the rejections in light of the amendments and following remarks.

Summary of Rejections

Claims 1 to 56 are rejected as follows:

- claims 1 to 56 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly non-enabled;
- claims 25, 26, 33, 54, and 56 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite;
- claims 1 to 26 are rejected under 35 U.S.C. § 102(a) as allegedly lacking novelty over Childers, *Drugs of the Future* ("Childers reference");
- claims 1 to 26 are rejected under 35 U.S.C. § 102(b) as allegedly lacking novelty over Baudy, *J. Med. Chem.* ("Baudy reference");
- claims 1 to 26 are rejected under 35 U.S.C. § 102(b) as allegedly lacking novelty over EP-A-0,778,023;
- claims 1 to 26 are rejected under 35 U.S.C. § 102(b) as allegedly lacking novelty over Kinney, *J. Med. Chem.* ("Kinney reference");
- claims 1 to 26 are rejected under 35 U.S.C. § 102(b) as allegedly lacking novelty over US-A-5,240,946;
- claims 1 to 26 are rejected under 35 U.S.C. § 102(b) as allegedly lacking novelty over US-A-5,168,103;
- claims 1 to 9 and 26 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting over claims 26 to 29 of US 10/969,715;
- claims 27 to 55 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting over claims 11 to 28 of US 10/820,216;

- claims 27 to 56 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting over claims 1 to 95 and 104 to 108 of US 10/961,871; and
- claims 21 to 24 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting over claims 37 to 53 and 57 to 73 of US 10/267,159.

Amendments to Claims

Applicants are herein amending claims 33 and 56 to correct an obvious typographical error, *i.e.*, where one set of "{ }" is missing in the name. Applicants submit that no new matter is introduced by the amendments to the claims and is fully supported by the specification is originally filed, including original Example 3 that provides mass spectrometry data.

Rejection under 35 U.S.C. § 112, first paragraph

Claims 1 to 56 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly non-enabled. Applicants traverse the rejection.

More specifically, the Office alleges that claims 1 to 56 are not enabled because the specification fails to describe "how to use" the compositions for treating and preventing "any and all diseases and/or conditions associated with excitatory amino acid receptor activity" (page 3). Further, the Office alleges that the claims are not enabled because they encompass the "prevention" of disorders. (*Id.*)

With respect to the prevention of disorders, claims 20 and 49 specify that the intranasal compositions are useful in preventing tolerance to opiate analgesia. No evidence has been presented that there is any reason to doubt that a skilled artisan would doubt that the intranasal compositions of the invention would not be useful in preventing such tolerance, especially in light of the fact that NMDA receptor antagonists are known to prevent the opiate analgesia tolerance. See, for example, the Trujillo abstract (enclosed). Medical

professionals have means to measure tolerance to opiate analgesia and would have no difficulty administering the compositions of the invention to effect the desired result, *i.e.*, prevention of the tolerance. No other evidence has been presented that establishes that a skilled artisan would doubt the use of the compounds of the invention, which are NMDA receptor antagonists, would not be useful in the treatment of the listed diseases and conditions.

Applicants submit that the composition claims meet the enablement requirements under 35 U.S.C. § 112, first paragraph. Applicants have described in sufficient detail to enable a person ordinary skilled in the art to make and use the intranasal compositions without undue experimentation. See, for example, applicants provide a description of the synthesis of the active compound on page 12, line 8 to page 14, line 3, three actual examples of how to formulate the intranasal compositions (Examples 1 to 3), and they also describe how other types of intranasal may be prepared on page 14, line 4 to page 19, line 8. Thus, applicants submit that the composition claims are enabled.

Further, applicants submit that the method of treatment claims also meet the enablement requirements under 35 U.S.C. § 112, first paragraph. With the exception of cerebral ischemia, it appears that the Office is challenging that there is a correlation between antagonists of the NMDA receptor and the treatment of the various diseases and conditions claimed. As applicants explained on page 14, line 8 to page 15, line 11, the present invention provides methods for treating conditions associated with glutamate abnormalities, *i.e.*, conditions produced by a disease or a disorder in which glutamate, typically in increased amounts, is implicated as a contributing factor. The Office has provided no evidence that any of the listed conditions would not be expected to be treated by the intranasal compositions of the invention. While the Office asserts that the methods includes diseases and/or conditions "not even known at this time," each of the method claims names specific conditions and does not, in fact, claim "all diseases and/or conditions associated with excitatory amino acid receptor activity," as suggested on page 3 of the Office Action.

Applicants are submitting herewith a number of review articles that show that there is recognized correlation between antagonism at the NMDA receptors and the specified diseases and conditions set forth in the claims:

- Wood PL.
  The NMDA receptor complex: a long and winding road to therapeutics.
  IDrugs. 2005 Mar;8(3):229-35. Review.

- Heresco-Levy U.
  Glutamatergic neurotransmission modulators as emerging new drugs for schizophrenia.
  Expert Opin Emerg Drugs. 2005 Nov;10(4):827-44. Review.

- Bergink V, van Megen HJ, Westenberg HG.
  Glutamate and anxiety.
  Eur Neuropsychopharmacol. 2004 May;14(3):175-83. Review.

- Parsons CG.
  NMDA receptors as targets for drug action in neuropathic pain.
  Eur J Pharmacol. 2001 Oct 19;429(1-3):71-8. Review.

- Brown DG, Krupp JJ.
  N-methyl-D-aspartate receptor (NMDA) antagonists as potential pain therapeutics.
  Curr Top Med Chem. 2006;6(8):749-70

- McCulloch J.
  Excitatory amino acid antagonists and their potential for the treatment of ischaemic brain damage in man.
  Br J Clin Pharmacol. 1992 Aug;34(2):106-14. Review.

Accordingly, applicants submit that claims 1 to 56 meet the enablement requirement under 35 U.S.C. § 112, second paragraph, and therefore request withdrawal of the rejection.

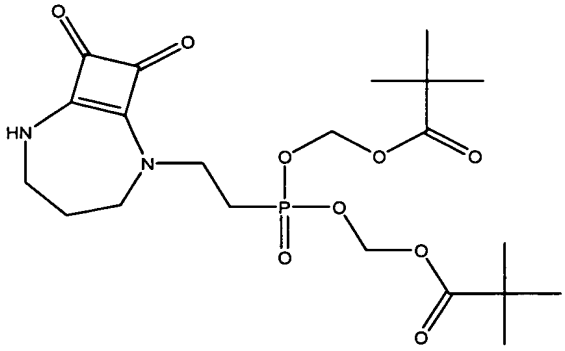
Rejection under 35 U.S.C. § 112, second paragraph

Claims 25, 26, 33, 54, and 56 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Applicants traverse the rejection.

Claims 25 and 54 are rejected for using the phrase "pain relieving agent." Applicants traverse the rejection because a skilled artisan would have no difficulty understanding the meaning of the phrase. Furthermore, on page 23, lines 22 to 25 and page 24, lines 10 to 25, applicants have provided numerous specific examples of pain relieving agents, leaving no doubt with the skilled artisan to the metes and bounds of the invention with respect to the pain relieving agents.

Claims 26 is rejected as allegedly vague and indefinite for being a duplicate of claim 1. Applicants traverse this rejection because claim 1 does not include the limitation "in unit dosage form or multiple dose form" and, accordingly, is different than claim 26 that contains this limitation.

Claims 33 and 56 are rejected because there is allegedly insufficient antecedent basis for "2,2-dimethyl propionic acid" in species c). Applicants are herein amending claims 33 and 56 to supply missing "{ }." The structure of the amended species c) is shown below and clearly have proper antecedent basis.



2,2-dimethyl-propionic acid{(2,2-dimethyl-propionyloxymethoxy)-[2-(8,9-dioxo-2,6-diaza-bicyclo[5.2.0]-non-1(7)-en-2-yl)-ethyl]-phosphinoyloxy} methyl ester

Applicants submit that claims 25, 26, 33, 54, and 56, as amended, meet the definiteness requirement under 35 U.S.C. § 112, second paragraph, and therefore request withdrawal of the rejection.

Rejections under 35 U.S.C. § 102

Childers Reference

Claims 1 to 26 are rejected under 35 U.S.C. § 102(a) as allegedly lacking novelty over the Childers reference. Applicants traverse the rejection.

The Childers reference discloses [2-(8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl] phosphonic acid (EAA-090) that is intraveneously administered (page 634, second column, page 635, first and second columns, and page 636, first column). No compositions are disclosed having EAA-090 and ***additives suitable for forming a composition for intranasal administration nor is there any reference to intranasal administration of the EAA-090***. Since the Childers reference does not disclose each and every element of the claim, the Childers reference does not anticipate claims 1 to 56. Accordingly, applicants request withdrawal of the rejection under 35 U.S.C. § 102(a) over the Childers reference.

Baudy Reference

Claims 1 to 26 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Figure 4 of the Baudy reference (*J. Med. Chem.*, 2001, 44, 10)[1] Applicants traverse the rejection.

Figure 4 of the Baudy reference discloses EAA-090 and a series of 2-amino-(phosphonoalkyl)-1H-benzimidazole-2-alkanoic acids. The compounds in the series (but not EAA-090) were administered intraperitoneally (page 1528, second column). No compositions are disclosed having EAA-090 and additives suitable for forming a composition for intranasal administration nor is there any reference to intranasal administration of the EAA-090 or 2-amino-(phosphonoalkyl)-1H-benzimidazole-2-alkanoic acids. Since the

[1]Applicants believe that the Office is referring to the correct Baudy reference, since there is a second Baudy reference from the *J. Med. Chem.*, 1993, 36, 3 on the PTO Form 1449.

Baudy reference does not disclose each and every element of the claim, the Baudy reference does not anticipate claims 1 to 56. Accordingly, applicants request withdrawal of the rejection under 35 U.S.C. § 102(b) over the Baudy reference.

EP-A-0,778,023

Claims 1 to 26 are rejected under 35 U.S.C. § 102(b) as allegedly lacking novelty over EP-A-0,778,023. Applicants traverse the rejection.

The Office references page 3, lines 9 and 10 as anticipating the composition and methods of the claimed invention. However, these lines do not relate in any way to the claimed invention. Applicants believe that the Office intended to refer to **[0009]** and **[0010]** of EP-B1-0,778,023. Nonetheless, this passage only discloses [2-(8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl] phosphonic acid (EAA-090) and other NMDA antagonists. It is disclosed that the compounds of the invention (rapamycin and NMDA or AMPA antagonist) may be administered orally or rectally. See **[0025]**, **[0028]** and **[0029]**. It is further disclosed that can be administered via "intramuscular, intraperitoneal, or subcutaneous injection" or intravenously. See **[0028]**. Finally, it is disclosed that rapamycin may be administered via intranasal or intrabronchial inhalation or insufflation. See **[0029]**. Notable, EP-B1-0,778,023 is silent with respect to intranasal administration or compositions of the NMDA antagonists.

Since EP-B1-0,778,023 does not disclose each and every element of the claim, EP-B1-0,778,023 does not anticipate claims 1 to 56. Accordingly, applicants request withdrawal of the rejection under 35 U.S.C. § 102(b) over EP-B1-0,778,023.

Kinney Reference

Claims 1 to 26 are rejected under 35 U.S.C. § 102(b) as allegedly lacking novelty over the Kinney reference. Applicants traverse the rejection.

The Kinney reference discloses the design and synthesis of EAA-090 and derivatives thereof, which were intraveneously administered (page 239, first column). No compositions are disclosed having EAA-090 and ***additives suitable for forming a composition for intranasal administration nor is there any reference to intranasal administration of the EAA-090***. Since the Kinney reference does not disclose each and every element of the claim, the Kinney reference does not anticipate claims 1 to 56. Accordingly, applicants request withdrawal of the rejection under 35 U.S.C. § 102(b) over the Kinney reference.

US-A-5,168,103 and US-A-5,240,946

Claims 1 to 26 are rejected under 35 U.S.C. § 102(b) as allegedly lacking novelty over US-A-5,168,103 and US-A-5,240,946. Applicants traverse the rejection.

The Office references Example 8 as anticipating the composition and methods of the claimed invention. This example discloses the synthesis of [2-(8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl] phosphonic acid (EAA-090). While the patents disclose that the compounds of the invention may be administered either orally or parenterally, there is no disclosure with respect to compositions for intranasal administration containing EAA-090 and additives suitable for forming a composition for intranasal administration.

Since US-A-5,168,103 and US-A-5,240,946 do not disclose each and every element of the claim, these patents do not anticipate claims 1 to 56. Accordingly, applicants request withdrawal of the rejection under 35 U.S.C. § 102(b) over US-A-5,168,103 and US-A-5,240,946.

Obviousness-type Double Patenting Rejections

Claims 1 to 9 and 26 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting over claims 26 to 29 of US 10/969,715. Claims 27 to 55

are provisionally rejected on the ground of nonstatutory obviousness-type double patenting over claims 11 to 28 of US 10/820,216. Claims 27 to 56 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting over claims 1 to 95 and 104 to 108 of US 10/961,871. Finally, claims 21 to 24 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting over claims 37 to 53 and 57 to 73 of US 10/267,159.

Applicants request that these provisional rejections be held in abeyance until the identification of otherwise allowable subject matter.

Conclusions

Applicants request:

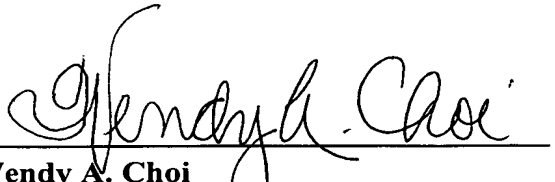
(1) entry of the amendments to the claims;

(2) reconsideration and withdrawal of the rejections of the claims; and

(3) allowance of claims 1 to 56.

If the Examiner is of a contrary view, the Examiner is requested to contact the undersigned attorney at (404) 459-5642.

Date: **September 6, 2006**



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Are NMDA receptors involved in opiate-induced neural and behavioral plasticity? A review of preclinical studies

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***Psychopharmacology (Berl)* 2000 Aug; 151(2-3):121-41**

ABSTRACT

RATIONALE: Research over the past decade demonstrating that NMDA receptor antagonists have the ability to inhibit opiate tolerance, sensitization and physical dependence has led to the suggestion that NMDA receptors may have a critical role in opiate-induced neural and behavioral plasticity. However, there have been suggestions that the effects of NMDA receptor antagonists on these phenomena result from non-specific behavioral or pharmacological effects, rather than from a specific inhibition of plasticity. OBJECTIVES: To review the literature in order to explore whether the effects of NMDA receptor antagonists on opiate-induced changes in behavior are best accounted for by an inhibition of neural and behavioral plasticity, or if alternative explanations might better account for the results. RESULTS: The effects of NMDA receptor antagonists on the development of tolerance to opiate analgesia and the development of opiate physical dependence do not appear to be due to confounding behavioral effects produced by high doses of NMDA receptor antagonists, "side-effects" of a particular drug or drug class, blockade of associative learning processes, or state-dependency. Results on tolerance and sensitization to the locomotor effects of morphine are more mixed and controversial; however, there is evidence suggesting that NMDA receptor antagonists may inhibit these phenomena in a similar manner. CONCLUSIONS: NMDA receptor antagonists appear to inhibit the neural plasticity underlying some forms of opiate tolerance, sensitization and physical dependence, suggesting that NMDA receptors are involved in the development of these drug-induced changes in behavior. Further research will help to determine the neural mechanisms responsible for these phenomena, and the therapeutic potential for drugs acting on the NMDA receptor complex in the treatment of pain and addiction.

Pain
Memantine
Zero tolerance?
NMDA antagonists
Pain and the NMDA receptor
NMDA antagonists for drug users
NMDA antagonists against morphine tolerance
NMDA antagonists, opioid receptors and tolerance

The NMDA receptor complex: A long and winding road to therapeutics

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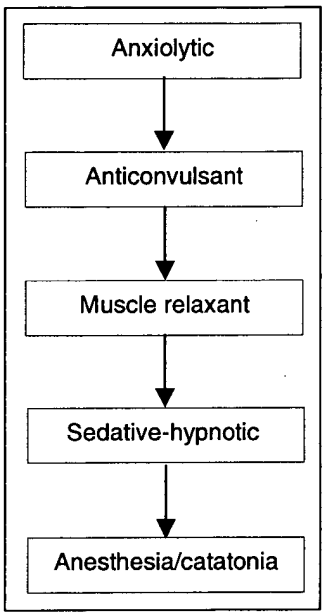
Advances in our basic understanding of inhibitory and excitatory amino acid neurotransmission have provided the foundation for directed drug discovery programs to modulate inhibitory GABAergic and excitatory N-methyl-D-aspartate (NMDA) receptor-mediated synapses. γ-Amino butyric acid ($GABA_A$) and NMDA receptors are complex ion channels formed by multiple protein subunits that act as binding sites for transmitter amino acids and as allosteric regulatory binding sites to regulate ion channel activity. In the case of the NMDA receptor complex, one such allosteric site binds the obligatory glycine and/or D-serine co-agonist. Historical data from preclinical and clinical studies of GABAergic agents have clearly demonstrated that direct receptor modulators lack sufficient therapeutic indices to warrant clinical utility. However, pharmacological modulation of allosteric sites of the GABA multimeric receptor has resulted in the clinical development of safe and efficacious agents, exemplified by the benzodiazepines. Research has also revealed a similar outcome for the NMDA receptor, with allosteric modulators demonstrating improved safety profiles in the modulation of excitatory amino acid (EAA) transmission compared with direct NMDA receptor antagonists. First-generation EAA drugs were low affinity channel blockers of the NMDA multimeric receptor complex and included the anesthetic agent ketamine and the Alzheimer's drug memantine. As predicted by preclinical studies, direct NMDA receptor antagonists (eg, selfotel (Novartis AG)) and high-affinity channel blockers (eg, dizocilpine) failed in the clinic as a result of narrow therapeutic indices. More recent efforts have focused on glycine/D-serine co-agonist function. These approaches include partial glycine agonists, in their agonist dose-range, for cognitive improvement and for treating schizophrenia. Such partial glycine agonists are also being advanced for the treatment of neuropathic pain in the antagonist dose range. An alternate approach to partial glycine agonists is to inhibit the uptake carrier(s) for glycine (ie, GlyT-1 and GlyT-2), thereby potentiating the lifetime of synaptic glycine. A number of glycine uptake inhibitors have been reported and their preclinical profiles support investigation into their utility in treating schizophrenia.

Keywords Glycine partial agonists, GlyT-1 uptake inhibitors, neuropathic pain, NMDA receptor, schizophrenia, stroke

Scientific background

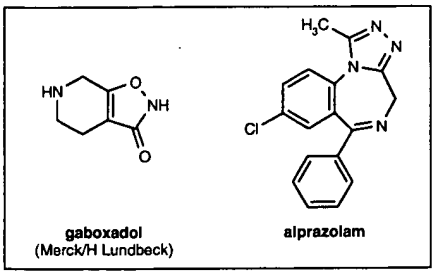
While there are a number of excitatory amino acid (EAA) receptor subtypes present in the central nervous system (CNS), the *N*-methyl-D-aspartate (NMDA) receptor subtype has received the greatest investment of resources with regard to drug discovery, largely because of its demonstrated roles in a vast array of CNS functions [1]. However, this wide range of CNS functions becomes a complicating issue when developing an NMDA receptor modulator that is both clinically efficient and lacks serious CNS side effects. Accomplishing this has been particularly challenging since significant disruptions of the dynamic balance between EAAs and the inhibitory amino acid neurotransmitter γ-amino butyric acid (GABA) result in a spectrum of CNS side effects that range from mild to serious. The overall pharmacological profile of positive modulators of the $GABA_A$ receptor and negative modulators of the NMDA receptor is a continuum, based on increasing drug concentrations (Figure 1).

Figure 1. Continuum of pharmacological actions of increasing doses of $GABA_A$ receptor agonists and NMDA receptor antagonists.



The complexity of developing safe pharmacological modulators of amino acid receptors has historically been well documented for GABA. For example, the search for positive modulators of this receptor led to the development of direct $GABA_A$ receptor agonists, such as gaboxadol (THIP, Merck & Co Inc/H Lundbeck A/S; Figure 2), however, the majority of these compounds failed upon reaching the clinic as a result of narrow therapeutic margins [2]. Alternatively, barbiturates, which are modulators of an allosteric site on the $GABA_A$ complex, demonstrated significantly improved therapeutic margins, although their use was limited due to issues concerning respiratory depression and abuse. Another class of allosteric modulators of the $GABA_A$ multimeric complex, the benzodiazepines (eg, alprazolam (Figure 2)), not only displayed superior therapeutic margins compared with direct agonists but also proved to be safe, except for a potential for abuse that was associated with chronic use (Table 1).

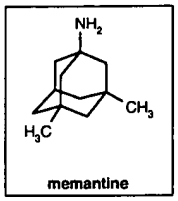
Figure 2. The structures of selected $GABA_A$ receptor modulators.



In the case of EAA systems, researchers have directed significant resources to discovering and developing NMDA receptor antagonists. In preclinical studies of NMDA receptor antagonists, a number of potential clinical limitations were revealed, for example: ataxia and catatonia; sedation; psychotomimetic actions and confusion; hypertension; and cerebral cortical vacuolization involving cytoplasmic vacuoles in limbic cortical, potentially leading to neuronal necrosis [3-5,6•,7].

As predicted by preclinical studies, and in analogy to $GABA_A$ receptor modulators, direct competitive NMDA receptor antagonists (Table 1) exhibited insufficient therapeutic margins for human use when evaluated in extensive clinical trials [8-10]. Similarly to the barbiturate modulation of the $GABA_A$ receptor, non-competitive NMDA receptor blockers demonstrated improved therapeutic margins and resulted in the introduction of several marketed products. These included ketamine, for anesthesia, and memantine (Figure 3), for the treatment of memory loss associated with Alzheimer's disease (AD) [11•], both of which are low-affinity, non-competitive inhibitors of ion channel function in the NMDA receptor complex. In contrast, high-affinity non-competitive NMDA receptor antagonists, like competitive receptor blockers, were limited by pyschotomimetic side effects (eg, agitation, abnormal dreaming, paranoia and hallucinations) [9,10]. The side effect of hypertension [9,10] appeared to be confined to high-affinity non-competitive NMDA receptor antagonists, and cortical vacuolization [7] was reported for both competitive and high-affinity non-competitive NMDA receptor antagonists.

Figure 3. The structure of memantine.



Potential clinical advantages for glycine site modulators

The glycine binding site of the NMDA receptor complex is classified as a co-agonist site with affinity for the endogenous ligands glycine and D-serine [12,13]. As an allosteric site it has been extensively investigated as a potential target for generating drug candidates with improved safety profiles [1,14]. This led to the discovery and characterization of a number of competitive glycine antagonists and partial glycine agonists [14-16]. The advantage of partial glycine receptor agonists resides in their ability to block excessive NMDA function while also potentiating NMDA receptor function in the case of abnormally depressed NMDA-mediated neurotransmission. The partial glycine receptor agonists therefore represent the ideal profile for an NMDA receptor drug candidate and should not produce a large imbalance between GABAergic and EAA systems.

The improved safety profile of glycine receptor antagonists and partial glycine receptor agonists compared with competitive and high-affinity non-competitive NMDA receptor antagonists is reflected by: (i) lack of phencyclidine-like behavioral effects [17] and lack of stimulation of limbic psychotomimetic actions in humans [9,10]; (ii) lack of dopamine turnover [18], which translates into lack of

Table 1. Comparative table of $GABA_A$ and NMDA receptor modulators.

Therapeutic index	GABA receptor modulators	NMDA receptor modulators
Low	Direct agonists • Muscimol • Gaboxadol	Competitive antagonists • Selfotel • CPP
Intermediate	Allosteric modulators: Barbiturates • Phenobarbital • Secobarbital	Allosteric modulators: Non-competitive antagonists • Aptiganel (Oregon Health Sciences University/ CeNeS Pharmaceuticals Inc) • Dizocilpine
High	Allosteric modulators: Benzodiazepines • Alprazolam • Diazepam	Allosteric modulators: Glycine antagonists • Gavestinel • Licostinel Allosteric modulators: Glycine partial agonists • D-Cycloserine • L-687414 • NT-13

CPP 3-((±)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid.

Table 2. Selected examples of drugs that have demonstrated preclinical efficacy but have failed in the clinic.

Compound	Action	Reference for preclinical efficacy	Reference for clinical failure
Clomethiazole	$GABA_A$ modulator	[21]	[9,10]
Selfotel	Competitive NMDA receptor antagonist	[6•]	[9,10]
Aptiganel	Non-competitive NMDA receptor antagonist	[6•]	[9,10]
Gavestinel	Glycine receptor antagonist	[22]	[9,10]

psychotomimetic activity in humans [9,10]; and (iii) lack of cortical vacuolization [19,20]. With regard to these issues, the therapeutic areas of stroke, neuropathic pain and schizophrenia will be reviewed. NMDA receptor antagonists have failed as therapeutic agents for the indication of stroke, however, NMDA receptor modulators may still offer medical breakthroughs in the clinical areas of neuropathic pain and schizophrenia.

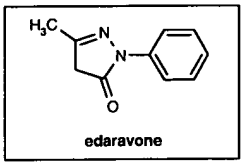
Clinical failure: Stroke

Both GABAergic compounds and NMDA receptor antagonists demonstrated neuroprotective potential against ischemic stroke in preclinical models, but failed in the clinic. Table 2 provides selected examples of such drug candidates.

While these failures indicated that NMDA receptor modulators will probably never be effective treatments for stroke, a substantial amount of data was generated defining the optimal preclinical criteria for a stroke therapeutic. These criteria include: efficacy in both the transient and permanent rat middle cerebral artery occlusion (MCAO) models of brain ischemia; a therapeutic window of ≥ 6 h in these models; verification of permanent functional recovery and not a transient delay in neuronal deficits; and significant safety margins to allow optimal dosing for intravenous infusions.

The only drugs to meet these criteria were not NMDA receptor modulators but were instead the antioxidant edaravone (Figure 4), which was launched in Japan in June 2001 [23], and the spin-trap (regenerating free radical scavenger) cerovive (AstraZeneca plc/Renovis Inc), which is currently in international phase III clinical trials. Futhermore, cerovive protected mitochondrial integrity in rat models of focal cerebral ischemia, hence maintaining energy reserves and blocking activation of the caspase cascade associated with efflux of cytochrome C from compromised mitochondria [24].

Figure 4. The structure of edaravone.



Potential clinical utilities

Neuropathic pain

NMDA receptor antagonists inhibited noxious sensory transmission in the dorsal horn of the spinal cord. These actions were demonstrated in a number of preclinical analgesia assays, initially with competitive NMDA receptor antagonists and then subsequently with non-competitive NMDA receptor antagonists [25,26]. However, this inhibition was consistently observed at doses that were also ataxic. The lack of therapeutic margin does not appear to be an issue with the low-affinity non-competitive NMDA receptor antagonist memantine, which demonstrated efficacy in both preclinical pain models [25,26] and in clinical trials with patients experiencing neuropathic pain [27].

Glycine receptor antagonists, such as L-701324 (Figure 5) and MDL-29951 (sanofi-aventis; Figure 5), and partial glycine receptor agonists, such as D-cycloserine (DCS), L-687414 and HA-966 (all Figure 6) were active in neuropathic pain models and also demonstrated superior therapeutic margins in relation to competitive and high-affinity non-competitive NMDA receptor antagonists (Table 3).

Figure 5. The structures of selected glycine antagonists.

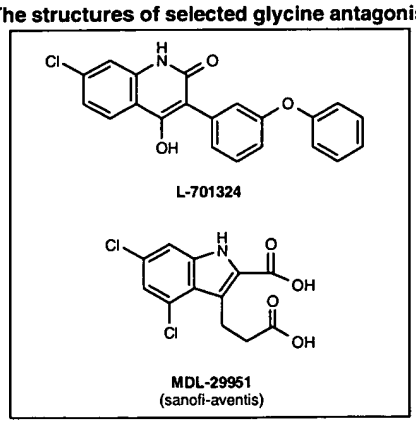
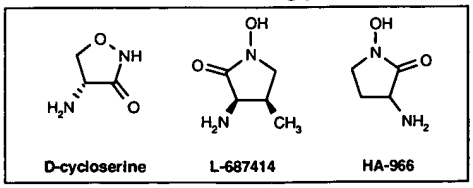


Figure 6. The structures of selected glycine partial agonists.



DCS is a broad-spectrum antibiotic, usually administered at a dose of 250 to 500 mg twice-daily for the treatment of tuberculosis. At much lower doses, DCS is also a potent NMDA receptor modulator. Preclinical data with DCS in rat models revealed a narrow glycine agonist dose range (1 to 10 mg/kg sc) and a wide glycine antagonist dose range above these concentrations [32]. In clinical trials, DCS

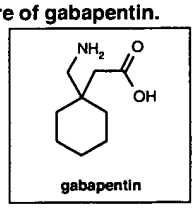
Table 3. Selected examples of glycine receptor antagonists and partial glycine receptor agonists.

Glycine receptor antagonists	Glycine receptor partial agonists
• L-701324 [28,29] • MDL-29951 [29] • 5,7-Dichloro-2-dihydroxy-3-phenyl-quinoline dione [29]	• D-Cycloserine [29] • HA-966 [29,30] • L-687414 [28,29] • NT-13 [31]

was administered twice-daily to AD patients at doses of 5, 15 or 50 mg, with results demonstrating that the 15-mg dose (0.2 mg/kg) improved implicit memory in patients [33]. These data were not substantiated in follow-up clinical trials [34]. However, these were difficult clinical trials to conduct since the agonist dose range of a partial glycine agonist (eg, DCS) is narrow and may not be wide enough for dosing a heterogeneous patient population, as in AD studies. The use of partial agonists in their antagonist dose range is much simpler with regard to both clinical design and dose-ranging. Currently, NT-13 (Nyxis Neurotherapies Inc) [31], a peptide (Thr-Pro-Pro-Thr), appears to be the only partial glycine agonist entering the clinic to demonstrate potential for a neuropathic pain indication.

The leading product for the treatment of neuropathic pain is the anticonvulasant gabapentin (Figure 7), which was launched by Pfizer Inc for the treatment of epilepsy and is currently registered for the indication of neuropathic pain [35]. The exact mechanism of analgesic action of gabapentin is not yet known, however, in neuropathic pain models (formalin late-phase response [36-38], substance P-induced thermal hyperalgesia [39,40] and tactile hyperalgesia with thermal injury [41]), the analgesic effects of the drug were dose-dependently antagonized by D-serine, an agonist of the NMDA-associated glycine receptor. This suggested that the analgesic actions of gabapentin in these models were dependent upon decreases in glycine-mediated NMDA receptor neurotransmission. Since gabapentin has no affinity for the various sites on the NMDA receptor, the compound most likely acts upstream of the NMDA receptor in a series of interlinked actions that ultimately require decreased NMDA-mediated neurotransmission for analgesic efficacy.

Figure 7. The structure of gabapentin.



gabapentin

Although gabapentin has demonstrated clinical efficacy in the treatment of neuropathic pain, the reduction in pain score is generally 2.05 points on an 11-point numerical rating scale [42]. It is expected that a direct modulator of the NMDA-associated glycine site might offer greater efficacy and possibly a superior therapeutic index to gabapentin. NT-13, which is entering phase I clinical trials, will be the first to test this hypothesis.

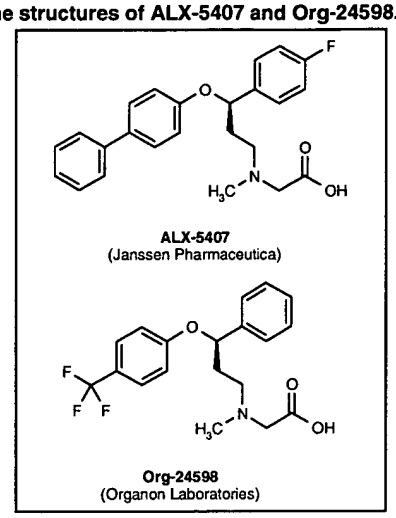
Schizophrenia

A decrement in EAA tone within limbic cortical regions has been hypothesized to play a role in the etiology of schizophrenia, based on data from preclinical studies of actions of antipsychotic agents in models of EAA hypofunction and data from studies of the clinical psychosis induced by non-competitive NMDA receptor blockers in humans [43-48]. Early research to potentiate NMDA-mediated neurotransmission has focused on the application of partial glycine agonists because these compounds would lack 'excitotoxic' potential. Indeed, since partial glycine agonists cannot induce full efficacy at the co-agonist site they offer a significant intrinsic safety feature. Furthermore, if excessive NMDA-mediated transmission were present at a given synapse, the partial glycine agonist would act as an antagonist and decrease NMDA receptor tone to reach the physiological range. Following the identification of the co-agonist site on the NMDA receptor, a plethora of publications negated the importance of this binding site, particularly as the micromolar concentrations of glycine observed in the cerebrospinal fluid were proposed to be capable of saturating glycine receptors *in vivo*. Unfortunately, these publications did not consider the rich literature history that establishes the principles of metabolic compartmentation within the synaptic cleft, which is key to limiting neurotransmission and maintaining point-to-point communication in the CNS. A review of the *in vivo* behavioral and neurochemical data that rebutted these misconceptions was first published in 1995 [49•] and the fact that the co-agonist site is not saturated *in vivo* has since been further validated using electrophysiological approaches [50,51]. The methods that have been investigated with the intention of potentiating NMDA neurotransmission in schizophrenia patients have included oral treatment with the partial glycine receptor agonist DCS [52,53], oral loading with glycine [53,54] or D-serine [55] and oral treatment with the glycine uptake inhibitor sarcosine [56].

These techniques appeared to provide clinical benefit with regard to negative symptoms and cognitive deficits that are observed in schizophrenia patients, except when used in combination with clozapine therapy [57]. The full benefit of this new approach requires more specific and/or more potent drugs for clinical testing. In this respect, extensive research into glycine uptake inhibitors is ongoing, with both glycine transporter type 1 (GlyT-1; ie, astrocytes) and GlyT-2 (ie, axons and presynaptic terminals) cloned and characterized [58]. Additionally, potent inhibitors of GlyT-1 (eg, ALX-5407 (Janssen Pharmaceutica NV; Figure 8)) [59-65] and GlyT-2 (eg, Org-24598 (Organon Laboratories Ltd; Figure 8)) [66,67] have been reported. The drawbacks to these first-generation glycine uptake inhibitors are their poor pharmaceutical properties and their potential to

increase glycine concentrations in both Glya and Glyb synapses, but the net consequences of this lack of specificity remain to be examined in more detail.

Figure 8. The structures of ALX-5407 and Org-24598.



Conclusion

In the last 20 years extensive resources have been devoted to characterizing the various components of the NMDA macromolecular receptor complex. The overall result of these efforts has led to the introduction of several low-affinity non-competitive NMDA receptor antagonists into clinical use. Future drug candidates appear to reside with the development of partial glycine agonists and glycine uptake inhibitors for the potential treatment of neuropathic pain and schizophrenia. For a recent review of the potential utility of NMDA receptor subtype modulators see reference [68].

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Review

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Glutamatergic neurotransmission modulators as emerging new drugs for schizophrenia

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Schizophrenia is a neurodevelopmental mental disorder whose aetiology includes genetic and environmental factors. Because of its early onset, chronicity and characteristic interference with education, employment and socialisation, this illness represents a tremendous human and economic burden to those who suffer from it, their families and society as a whole. Conventional and atypical antipsychotics, which mainly affect dopaminergic and serotonergic neurotransmission, are currently the cornerstone of schizophrenia treatment. Although the introduction of atypical antipsychotics represents a major development and, overall, antipsychotics are efficacious against psychotic symptoms, there remains a critical unmet need for innovative medications with improved efficacy and tolerability for the negative symptoms and cognitive deficits associated with schizophrenia. These dysfunction domains are reliable predictors of long-term disability and treatment outcome and are presently viewed as crucial targets for new pharmacological treatments of schizophrenia. Within this medication development framework, the modulation of glutamatergic neurotransmission has become the focus of intense research. Glutamate (GLU)-mediated neuronal processes are critical throughout the brain and glutamatergic neurotransmission dysfunctions have been hypothesised to play a crucial role in schizophrenia pathophysiology. Glutamatergic neurotransmission may be modulated at multiple levels, with GLU receptor families and their subtypes representing a modulatory site-rich environment for drug research. Numerous types of neurotransmission modulators, acting at the NMDA, AMPA and metabotropic GLU receptors, and/or affecting GLU synaptic release, are hypothesised to be beneficial for schizophrenia treatment, and are presently in various stages of development. For some of these compounds, preliminary studies have furnished encouraging clinical data. Ongoing and planned research is expected to provide, in the near future, critical information regarding the practical utility and tolerability of glutamatergic approaches for schizophrenia pharmacotherapy.

Keywords: AMPAkines, cognitive deficits, GLU-release inhibitors, mGluR modulators, negative symptoms, NMDA receptor modulators, schizophrenia

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1. Background

Schizophrenia, which has long been considered the most chronic, debilitating and costly mental illness, is one of the most devastating human diseases, ranking among the top 10 causes of disability in developed countries worldwide. It affects ~ 1% of the world's population and generally manifest in late adolescence or early adulthood [1]. The core phenomenological features of schizophrenia include patterns of myriad symptoms, most of which fall into categories termed 'positive,' 'negative' and 'cognitive.' Positive symptoms generally imply awareness beyond normal experience;

negative symptoms generally reflect diminished experience. Cognitive or 'disorganised' symptoms refer to deficits in maintaining attention, perception, learning, memory and thinking on an abstract level. The positive symptoms of schizophrenia (e.g., agitation, delusions, hallucinations and grossly disorganised behaviour), being more easily identified and more likely to lead to hospitalisation, have been traditionally used as main determinants of patient outcome. However, the negative and cognitive components, although less florid, are usually much more pernicious. The negative symptoms are deficiencies in vital areas of human endeavour, including motivation, verbal and nonverbal communication, interest in socialisation, experiencing pleasure and expression of affect. They have a deleterious effect on performance at school or work and a destructive impact on relationships with friends and family, which usually leads to a removal of both focus and potential support networks from an individual's life. Thus, negative symptoms are consistently found to be predictors of family and community functioning, especially when compared with positive symptoms [2]. Intertwined with the negative/positive symptomatology, neurocognitive testing usually reveals a pattern of deficits suggestive of widespread dysfunction. Virtually all aspects of brain function, from basic sensory processes to the most complex aspects of thought, are affected to some extent. Overall, the widespread cognitive dysfunction characteristic of schizophrenia represents one of the main prognostic factors associated with the illness, and is presently considered a reliable predictor of long-term disability and treatment outcome [3].

The course of schizophrenia varies, but, despite treatment, most patients have a chronic course with frequent psychotic relapses characterised by exacerbation of positive symptoms and rehospitalisation. Most affected individuals have substantial lifelong impairment and more than half require continuous support, whether living in the community or in long-term institutions. Some 15% of patients reside for long periods in chronic mental health facilities and another 15% end up incarcerated for petty crimes and vagrancy. The standardised mortality ratio for persons with schizophrenia is significantly higher than in the general population, reflecting higher rates of both unnatural and natural death. The high prevalence of obesity, smoking and alcohol abuse in persons with schizophrenia contributes to this increased risk. The suffering of people with schizophrenia is further highlighted by a 9 – 13% lifetime risk of committing suicide, estimated to be 20- to 50-times higher than that of the general population. Overall, the economic costs imposed by schizophrenia on society are enormous. More than a third of mental hospital beds are occupied by schizophrenia patients. Direct and indirect costs for the treatment of this illness are estimated at $20 – 35 billion annually in the US alone; when the costs of lost production are included the estimated costs are > $46 billion [4].

Twin studies provide evidence of a genetic contribution to schizophrenia: if one identical twin has the disease, the other has an ~ 30 – 40% chance of developing it, even if the two have been brought up in different families. Nevertheless, the shared genes of identical twins are not sufficient to give rise to the disease in all instances, thus pointing to the involvement of additional factors [4]. On the other hand, the existence of multiple predisposing variants of genes (i.e., alleles) for schizophrenia may help explain the variability of symptoms across patients, reflecting perhaps, for different individuals, predominant effects on different brain neurotransmitter systems [5].

During the last 10 – 15 years, within the framework of the recognised importance of genetic vulnerability, the concept of schizophrenia as a 'functional' psychosis has changed to the current paradigm of schizophrenia as a neurodevelopmental disorder. The onset of the illness with substantial progression of symptoms during the first few years and later stabilisation, makes schizophrenia very unusual among brain disorders. The fact that most cases arise in early adulthood is also an important clue with respect to pathophysiology because developmental changes may continue to occur in the brain during this period. The neurodevelopmental hypothesis (NDH) of schizophrenia basically suggests that a disruption of brain development during early life underlies the later emergence of illness during adulthood, while neuropathological processes may contribute to deterioration and illness progression. Recent versions of the hypothesis have incorporated evidence from structural neuroimaging studies, which suggests changes in brain volumes after the onset of schizophrenia. More detailed models indicating that multiple insults are required over the lifespan rather than one single early-life event have replaced early versions of the NDH, which were based on a 'static encephalopathy' concept [6]. One of the most important conclusions stemming from recent research is that no single brain area is 'responsible' for schizophrenia and that there is no pathognomonic neuroanatomical or neuropsychological profile of schizophrenia, which probably reflects the aetiological heterogeneity within this disorder [7]. This concept is supported by evidence suggestive of defects in interneuronal connectivity in the frontal and temporal cortical and related subcortical regions of the brains of individuals with schizophrenia [4-7].

The cornerstone of schizophrenia treatment remains pharmacotherapy, which presently employs antipsychotic medications [8]. Historically, the principles guiding antipsychotic drug development have massively influenced the understanding of schizophrenia, while simultaneously undergoing a reshaping process in view of the emerging complexities associated with the biological foundation of this illness. For decades, theories of schizophrenia have focused on a single brain neurotransmitter, dopamine (DA), based primarily on the observation that all conventional antipsychotic drugs are characterised by antagonistic activity at DA D2 receptors and degrees of therapeutic efficacy that highly correlate with their affinity for striatal D2 receptors [9]. Over the past 15 years, a fundamental paradigm change – substantial, but incomplete, attenuation of D2 receptor function combined with blockade

or inverse agonism of serotonin 5-HT_2 receptor function – has furnished the theoretical background for the development of atypical, second-generation antipsychotics (SGAs) [10]. However, despite the significant progress made during the last decades, for most patients there is still an urgent need for the development of novel treatment strategies. Accumulating evidence indicates that it is highly unlikely that the constellation of symptoms that characterise schizophrenia may reflect dysfunctions of single neurotransmitter systems. Consequently, the development of novel antipsychotic drugs now takes place within the framework of a multifactorial hypothesis of schizophrenia aetiology in which, besides DA, additional neurotransmitters are implicated, and neurotransmitter interactions in complex neurocircuits systems are highlighted [11]. It is hoped that these efforts may lead to new pharmacological treatment modalities and improved compounds, specifically efficacious against schizophrenia symptom clusters.

Within this medication development framework, the modulation of glutamatergic neurotransmission has become the focus of intense research. Glutamate (GLU) is the primary excitatory neurotransmitter in the mammalian brain. Unlike DA, which plays an important role only in isolated regions, GLU-mediated functions are critical throughout the brain. Approximately 60% of neurons in the brain, including all cortical pyramidal neurons and thalamic relay neurons, utilise GLU as their primary neurotransmitter. As a result, virtually all corticofugal, corticocortical and thalamocortical neurotransmission in the brain is mediated by GLU. In light of the major role of glutamatergic pathways in the modulation of mood, cognitive processes and motor behaviour, their reciprocal interactions with monoaminergic networks and their intense innervation of corticolimbic structures and the basal ganglia, it is thus reasonably expected that pharmacological modulation of glutamatergic neurotransmission would be highly relevant to schizophrenia treatment.

GLU receptors are divided into two broad families. Ionotropic receptors are differentiated based on sensitivity to the synthetic GLU derivatives NMDA, AMPA and kainic acid (KA). Metabotropic glutamatergic receptors (mGluRs), which are G protein-coupled and mediate longer-term neuromodulatory effects of GLU, are divided into groups on the basis of effector coupling and ligand sensitivity. Each of the four classes of GLU receptors (NMDA, AMPA, KA and mGluRs) are derived from distinct gene families encoding a variety of subunits that can form various receptor/channel combinations with a broad range of characteristics [12]. These GLU receptor families and their subtypes represent a modulatory site-rich environment for drug research and have increasingly become, during the last decade, the molecular targets for the development of innovative therapeutic agents in schizophrenia.

2. Medical need

Treatment resistance in schizophrenia remains a complex mental health problem. The tremendous human suffering and costs that continue to be generated by this illness reflect, at least in part, the inadequacies of the arsenal of medications presently available. Overall, both conventional and the newer, atypical antipsychotics do not reduce psychotic symptoms in all patients and have limited efficacy against negative symptoms and cognitive deficits that are increasingly conceptualised as cardinal features of schizophrenia. Patients with persistent positive symptoms still account for 20 – 30% of the people who have chronic schizophrenia [13]. Furthermore, the limited clinical effectiveness of available treatments against negative and cognitive symptoms undermines further efforts to rehabilitate the patients and limit chronicity [14]. Meta-analytic techniques suggest that patients with schizophrenia perform 0.5 – 1 standard deviations below the normal mean in numerous areas of neurocognition [15]. Neurocognitive impairment is associated with key features of schizophrenia, such as the inability to acquire skills, poor social problem solving and poor community functioning [3,16], and may actually represent a stronger correlate of poor outcome than any other symptom domain [3]. Consequently, neurocognitive performance has come to be viewed as a crucial target for pharmacological treatments for schizophrenia.

An additional liability, long known to be associated with conventional neuroleptics and presently suggested also in relation to some newer, atypical antipsychotics, is the induction of various types of side effects that may interfere with treatment, lead to decreased compliance and contribute to comorbidity and mental and physical disability. Thus, overall, for many patients there still is a great need for the development of treatment strategies that may offer improved tolerability and additional therapeutic benefits.

2.1 Existing treatments

Antipsychotic medications (Table 1) are the mainstay of pharmacological treatment for schizophrenia and psychotic disorders in general. At present, this type of medication includes conventional antipsychotics (or neuroleptics), which are also termed first-generation antipsychotics, and atypical antipsychotics, which are also termed SGAs. Additional types of psychotropic drugs used in schizophrenia as adjuvants to antipsychotics in order to increase efficacy or combat side effects, include lithium carbonate, anticonvulsants (e.g., carbamezepine, valproate), benzodiazepines and anticholinergic drugs.

Both clinical practice and research indicate that, in general, conventional antipsychotics do not adequately alleviate negative and affective symptoms and cognitive impairments [17]. A debate continues as to whether this type of drug can actually contribute to these symptom domains. A recent meta-analysis [18] reevaluates previous data and suggests that typical antipsychotics may provide modest-to-moderate gains in multiple cognitive domains, when compared with placebo. On the other hand, it has been highlighted that most studies showing brain shrinkage in schizophrenia were performed in patients receiving high D2 occupancy antipsychotic drugs, and recent longitudinal data of adult patients receiving newer, atypical

Table 1. Commonly used conventional and atypical antipsychotics.

	Generic name	Trade name	Year of US FDA approval
Conventional antipsychotics	Chlorpromazine	Thorazine	1953
	Perphenazine	Trilafon	1958
	Trifluoperazine	Stelazine	1958
	Fluphenazine	Prolixin	1959
	Thiothixene	Navane	1967
	Haloperidol	Haldol	1967
Atypical antipsychotics	Clozapine	Clozaril	1989
	Risperidone	Risperdal	1993
	Olanzapine	Zyprexa	1996
	Quetiapine	Seroquel	1997
	Ziprasidone	Geodon	2001
	Aripiprazole	Abilify	2002

antipsychotics suggest that no shrinkage occurs over time in these patients [19].

The spectrum of side effects induced by conventional antipsychotics extends beyond possible deleterious effects on schizophrenia symptomatology. Neuroleptic-induced extrapyramidal side effects (EPS) are characteristically associated with most conventional antipsychotics and may emerge in up to 75% of patients treated with these medications [20]. Additional debilitating side effects contribute to a high degree of noncompliance. Symptoms of pharmacogenic anhedonia, akinetic depression or neuroleptic-induced deficit syndrome are barely measurable on objective rating scales and are difficult to differentiate from the negative symptoms of schizophrenia [21]. Hyperprolactinaemia may result in galactorrhoea and sexual dysfunction, and weight gain can impact on a patient's self-esteem and increase the risk of patients developing a variety of disorders such as coronary heart disease or diabetes [22].

The introduction of atypical antipsychotics during the last two decades represents a major development in the treatment of schizophrenia and has provided basic and clinical data allowing for the steady evolution of the DA hypothesis of schizophrenia from one involving only DA receptors to one that includes interactions with multiple DA receptor subtypes (e.g., D3 and D4) and other neurotransmitter receptors. In general, atypical antipsychotics are considered at least as effective as classical neuroleptics in combating positive symptoms of schizophrenia. However, their claimed advantages for the treatment of negative symptoms and cognitive deficits are less established. Many studies comparing atypical antipsychotics with conventional antipsychotics have found the atypical agents to be superior for the treatment of negative symptoms, affective symptoms and cognitive deficits [23,24]. Nevertheless, it is a matter of debate whether the atypical agents have a direct effect on primary negative symptoms (i.e., those negative symptoms intrinsic to schizophrenia) or an indirect effect mediated by an improvement in the putative causes of secondary negative symptoms, such as medication side effects, inadequate social stimulation, unrecognised depression and/or intrusion of positive symptoms. Recent considerations, such as the importance of the dose of typical antipsychotic comparators, have called into question whether atypical antipsychotics actually improve cognition or whether they simply afford a release from the deleterious effects, such as EPS, of inappropriately large doses of typical antipsychotics (usually haloperidol) and concomitant adjunctive agents such as anticholinergics [23,24].

The most solidly demonstrated and recognised advantage of SGAs is that they are associated with a lower liability for EPS and a reduced risk of tardive dyskinesia [25,26]. This improved tolerability profile is probably one of the main factors guiding present shifts from conventional to atypical antipsychotics in overall antipsychotic drugs consumption. However, SGAs are not devoid of problematic side effects and clinical and research interest has recently focused on metabolic and endocrine dysfunctions that may be induced by some of the presently used atypical antipsychotics [27,28].

Clozapine, the first atypical antipsychotic, introduced in clinical practice in the early 1970s, probably represents the only presently available antipsychotic having a truly unique therapeutic profile. Clozapine is a unique prototype atypical, tricyclic, dibenzodiazepine-derivative antipsychotic agent. It has been proved to be effective and significantly superior to conventional neuroleptics in controlled studies in treatment-resistant schizophrenia, is presently the most effective antipsychotic for severely ill hospitalised patients and has been found to produce a very low incidence of motor side effects [29]. However, clozapine is associated with a relatively high risk for seizures, with potentially life-threatening agranulocitosis in 1 – 2% of patients, requiring long-term monitoring of the neutrophil count [30], and with significant weight gain [27,28]. These drawbacks weigh heavily when risks and benefits of clozapine use are analysed. For example, using Framingham

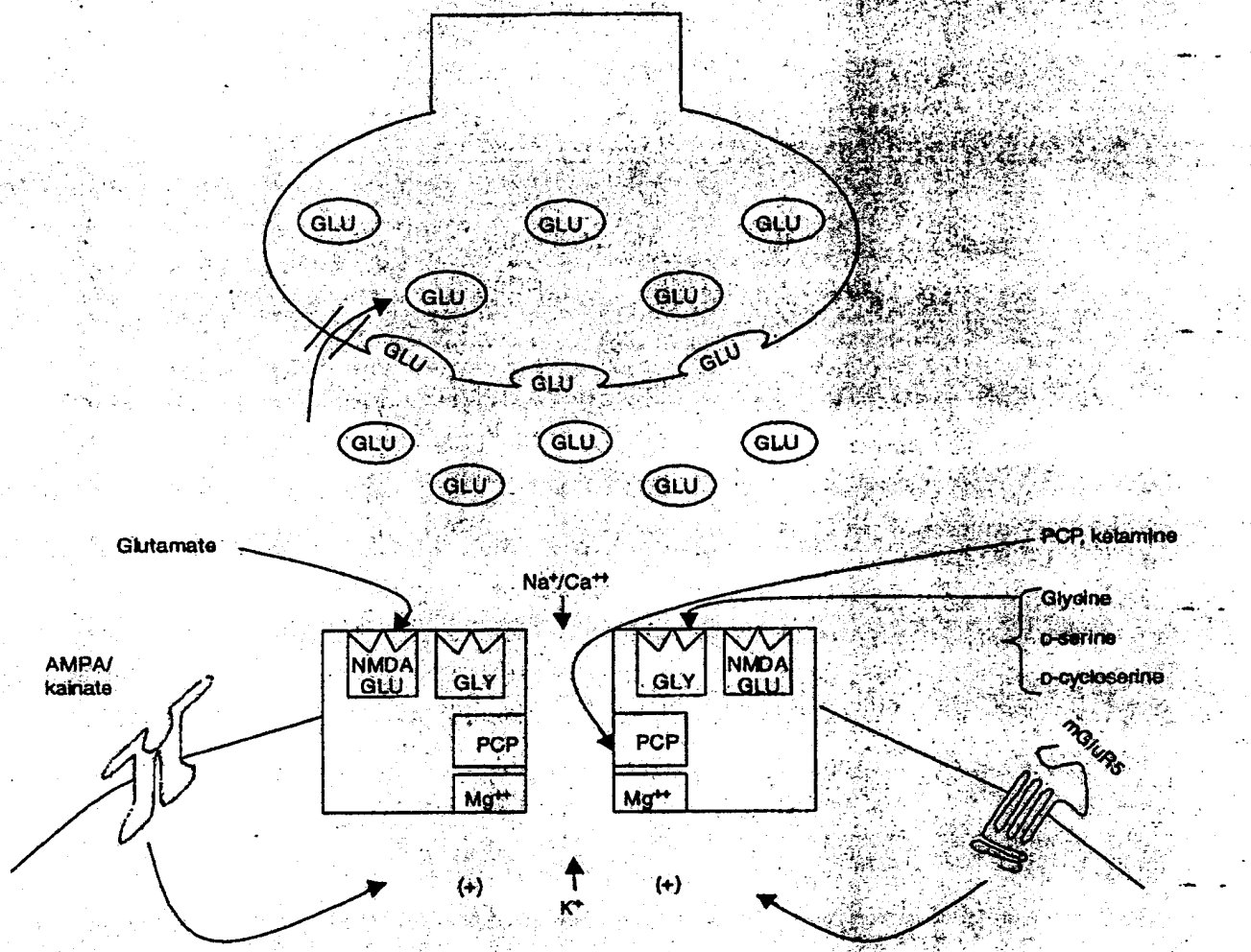


Figure 1. Simplified schematic diagram of a generic glutamatergic synapse. GLU is released from the presynaptic terminal and acts on ionotropic (NMDA, AMPA/kainate) and metabotropic (represented by mGluR5) GLU receptors. NMDA receptor is embedded in a postsynaptic structure that produces a tight coupling with other GLU receptors. Activation of AMPA/kainate and/or mGluR5 receptors can result in potentiation of NMDA receptor function. GLY, GLU/NMDA and PCP binding sites of the NMDA receptor are illustrated.
GLU: Glutamate; GLY: Glycine; mGluR5: Group 5 metabotropic GLU receptors; PCP: Phencyclidine.

Heart Study data, Fontaine *et al.* [31] estimated that although clozapine decreased suicidal behaviour in 492 of 100,000 schizophrenia patients over a 10-year period, the weight gain induced by clozapine would be expected to result in 416 additional deaths.

3. Current research goals

In view of the limitations of existing treatments and given the recent developments in the understanding of pathophysiological processes associated with schizophrenia, current research in the therapeutics of this illness focuses on the development of compounds characterised by mechanisms of action different than those of presently available antipsychotics.

The main clinical goals for the development of new therapeutic agents for schizophrenia include the following:

- superior efficacy, versus drugs used at present, against negative symptoms
- superior efficacy, versus drugs used at present, against cognitive deficits
- prevention of disease and disability progression
- preserved or improved efficacy against positive symptoms
- improved therapeutic index (i.e., less toxicity in relation to obtained benefits)
- avoidance of motor and/or metabolic side effects
- lack of detrimental interactions with other drugs, including presently used antipsychotics with which they may be coprescribed

It is hypothesised that beyond the traditional measures of symptomatology, achievement of these goals would necessarily lead to:

- increased levels of recovery
- decreased cumulative morbidity
- lower relapse rates
- better long-term outcome

4. Scientific rationale

Schizophrenia is currently the best established of the potential therapeutic targets for modulation of glutamatergic neurotransmission. NMDA, and also non-NMDA glutamatergic receptors, play an important role in a variety of cardinal brain functions, including memory and learning, synaptic and developmental plasticity, sensory information and coordinated movement patterns, which appear to be disturbed in schizophrenia [32]. Postmortem studies have identified abnormalities of GLU receptor density and subunit composition in the prefrontal cortex, thalamus and temporal lobe – areas that exhibit impaired activation during performance of cognitive tasks in schizophrenia. These findings suggest that glutamatergic dysregulation may occur in regionally specific subpopulations of glutamatergic receptors and support the potential value of a glutamatergic model for guiding research into the pathophysiology and treatment of schizophrenia. Glutamatergic receptor dysfunction could also play a role in neuroarchitectural abnormalities that have been described in schizophrenia, such as aberrant neuronal migration or reduced synaptic connection, due to the role of glutamatergic receptors in regulating neuronal migration, neurite outgrowth, synaptogenesis, and the 'pruning' of supernumerary neurons by apoptosis [5,6,33,34].

Because an extensive and functionally diverse range of GLU receptor subtypes are genetically encoded and can interact with environmental stressors during brain development, the model of glutamatergic dysfunction may account for the interplay of genetic and environmental risk factors identified in schizophrenia. Furthermore, dysfunction of glutamatergic neuronal systems is not inconsistent with the DA hypothesis of schizophrenia because reciprocal synaptic relationships between forebrain dopaminergic projections and glutamatergic systems have been well described. A relative increase in dopaminergic activity and/or a relative decrease in glutamatergic transmission could precipitate psychosis. This interaction between glutamatergic and dopaminergic systems suggests that the dopaminergic hyperactivity observed in schizophrenia may actually be secondary to deficits in glutamatergic neurotransmission [11].

The strongest line of evidence in support of an NMDA receptor hypofunction hypothesis of schizophrenia is based on the psychomimetic effects of phencyclidine (PCP), ketamine and other noncompetitive antagonists of NMDA receptor-mediated neurotransmission. These agents induce schizophrenia-like psychotic symptoms in normal volunteers and re-emergence of presenting symptoms in remitted patients. Unlike amphetamine, which stimulates DA release in the brain and can induce hallucinations and delusions in normal users, NMDA receptor antagonists can also induce negative symptoms, thought disorder and cognitive dysfunction similar to that observed in schizophrenia [5,10,33,34].

PCP and ketamine induce these behavioural effects by binding to the PCP site located within the ion channel associated with the NMDA receptor (Figure 1). Their binding to this site leads to noncompetitive blockade of NMDA receptor-mediated neurotransmission, indicating that endogenous NMDA receptor dysfunction may play a critical role in the pathophysiology of schizophrenia. Moreover, competitive NMDA receptor antagonists (e.g., 3-[2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid, CGS-19755) that block NMDA receptor by acting at the NMDA, rather than PCP recognition site, also appear to induce PCP-like effects in humans [35]. These findings are in agreement with the concept of NMDA receptor hypofunction in schizophrenia and led to the hypothesis that this phenomenon may not be PCP receptor-specific, but could result from a dysfunctional blockade of the NMDA receptor–ionophore complex [36]. The ability of NMDA receptor antagonists to induce schizophrenia-like symptoms and cognitive deficits suggests that enhancement of NMDA receptor-mediated neurotransmission may significantly ameliorate such symptom domains in schizophrenia.

It is also hypothesised that by facilitating NMDA receptor-related neuroplasticity, drugs that facilitate NMDA receptor function will increase the capacity of cortical networks to undergo experience-dependent modification [36]. This could be achieved either with compounds directly modulating NMDA receptor function (e.g., NMDA receptor agonists) or with modulators of other types of GLU receptors (i.e., AMPA and mGluRs) that may help increase the level of neural network activity and enhance the voltage-dependent recruitment of NMDA receptors (Figure 1) [37].

Alternative, but not mutually exclusive, glutamatergic models highlight the possible role of excessive GLU release and excitotoxicity in schizophrenia [10,12]. Following acute treatment, NMDA receptor antagonists stimulate prefrontal GLU release, which may independently induce schizophrenia-like impairment in cognitive performance [39]. NMDA receptor antagonists may also induce neurodegeneration of pyramidal neurons following acute or chronic administration. In this model, it is proposed that symptoms of schizophrenia do not reflect acute NMDA receptor blockade, but rather apoptotic changes that are associated with excessive GLU release and occur in susceptible brain regions, particularly frontocingulate areas [36]. Overall, these findings stress the importance of exploring the blockade of dysfunctional GLU release as an additional pharmacological strategy to be used in schizophrenia. Moreover, they suggest that regional imbalances and/or dysfunctional attempts to reach homeostasis may occur in schizophrenia. This hypothesis may be consistent with

Box 1. Types of glutamatergic neurotransmission modulators under development/evaluation for schizophrenia treatment.

NMDA receptor

- glycine site full agonists
- glycine site partial agonists
- glycine/D-serine transport inhibitors

AMPA receptor

- positive allosteric modulators (AMPAkines)

Metabotropic receptors

- group I metabotropic receptor (mGluR1, mGluR5) modulators
- group II/III metabotropic receptor modulators

Ion-channel blockers/glutamate release inhibitors

- lamotrigine (Lamictal®, GlaxoSmithKline)
- riluzole (Rilutek®, Aventis Pharma AG)

postmortem data indicating that the same individual with schizophrenia may manifest deficient glutamatergic innervation in one brain region and excessive innervation in another [38].

5. Competitive environment

Numerous neurotransmission modulators that act at diverse types of brain GLU receptors and/or affect GLU synaptic release are hypothesised to be beneficial in schizophrenia treatment. This section reviews the types of glutamatergic agents that are presently under development (Box 1) and highlights those compounds that have been assessed in clinical trials (Table 2).

5.1 NMDA receptor modulators

NMDA receptors are the most complex of the ionotropic GLU receptors and a primary drug development target for schizophrenia treatment. In addition to the recognition site for GLU, NMDA receptor contains a neuromodulatory site for glycine (GLY) that affects channel open time and desensitisation rate in the presence of agonist (GLU), but does not itself induce channel opening (Figure 1). As such, chronic GLY treatment in rodents has been found not to induce excitotoxicity [39,40]. NMDA receptors are blocked in a voltage sensitive manner by Mg^{2+} which binds to a site within the NMDA receptor ion channel. As a result, NMDA receptors are uniquely voltage- as well as ligand (GLU)-sensitive, which permits them to participate in multiple neurocognitive processes, including long-term potentiation, nonlinear amplification, coincidence detection and attentional gating [32].

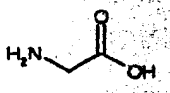
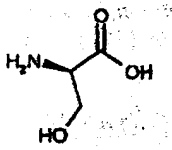
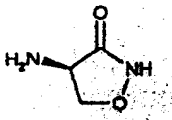
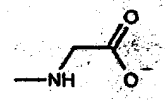
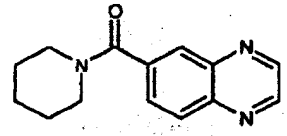
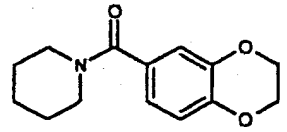
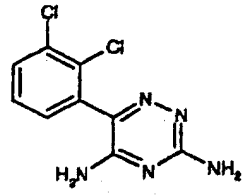
So far, the NMDA receptor site that has been explored most efficiently for drug development is the NMDA receptor-associated GLY site. A first-generation approach to potentiation of NMDA receptor-mediated neurotransmission *in vivo* has been the administration of the amino acids GLY and D-serine (DSR), which serve as endogenous modulators of the NMDA receptor complex. A more recent approach has been the targeting of amino acid transporters that regulate amino acid levels *in vivo*, analogous to use of selective serotonin reuptake inhibitors, rather than exogenous tryptophan administration to modulate brain serotonin levels in depression.

5.1.1 *NMDA receptor – GLY site agonists*

Clinical trials in schizophrenia have been conducted with the endogenous ligands GLY and DSR [101-104] that function as full agonists at the GLY site. Based on the known ability of these amino acids to stimulate NMDA receptor *in vitro* [12] it was predicted that additional GLY site ligands such as D-alanine or their precursors should also be effective in the treatment of schizophrenia [41,103]. Both GLY and DSR cross the blood–brain barrier and so can be administered systemically. GLY, however, is extensively metabolised in the periphery and large doses must be given to rodents (0.8 – 1.6 g/kg) and humans (0.4 g/kg i.v.; 0.8 g/kg p.o.) in order to significantly affect brain GLY levels [12,33,41]. DSR is nephrotoxic in rats; however, this effect is species-specific and does not seem to generalise even to other rodent species [42]. Physiologically, the GLY binding site appears to be approximately half-saturated under physiological circumstances, so that saturation of this site by exogenous compound or GLY/DSR transport inhibitors may lead to approximate doubling of NMDA receptor-mediated neurotransmission [12]. In addition to endogenous ligands, the synthetic GLY site agonist D-cycloserine (DCS) has been assessed clinically. DCS [105] is an antituberculosis drug that fortuitously crossreacts with the NMDA receptor GLY site. Although DCS crosses the blood–brain barrier readily, it functions only as a partial agonist, with 30 – 60% of the efficacy of GLY or DSR [12,33].

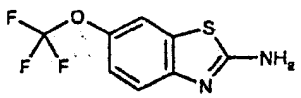
All clinical trials with GLY site agonists performed so far have employed these agents as adjuvants to ongoing treatment with conventional neuroleptics, clozapine or newer atypical antipsychotics (i.e., olanzapine and risperidone). Overall, GLY has been found to be effective at doses of 30 – 60 g/day (0.4 – 0.8 g/kg/day); DSR is effective at a dose of 2.1 g/day (30 mg/kg/day) and DCS at a dose of 50 mg/day. With both GLY and DSR, the effectiveness of higher doses has not been explored, so maximal benefit obtainable from GLY-site stimulation is unknown [12,33]. DCS was found to have a narrow therapeutic window: doses < 50 mg/day are ineffective, whereas doses > 100 mg/day cause symptom exacerbation due to emergent NMDA receptor antagonist effects [43]. In a retrospective comparison among schizophrenia patients who participated in controlled trials of both GLY and DCS, the degree of improvement was found to be significantly larger during GLY than DCS treatment on both an individual subject and group level [44]. Nevertheless, using functional magnetic resonance imaging, it was shown that patients receiving DCS, but not

Table 2. Competitive environment

Compound	Company	Structure	Stage of development	Mechanism of action
Glycine	Glytech		Phase II	NMDAR-GLY site full agonist
D-serine	Prestwick Pharmaceuticals		Phase II	NMDAR-GLY site full agonist
D-cycloserine (seromycin)	GD Searle		Phase II	NMDAR-GLY site partial agonist
Sarcosine (*N*-methylglycine)	Prestwick Pharmaceuticals		Phase II	GLYT1 inhibitor
CX-516	Cortex Pharmaceuticals Organon		Phase II	AMPA receptor positive modulator
CX-717*	Cortex Pharmaceuticals Organon		Phase II	AMPA receptor positive modulator
Lamotrigine (Lamictal®)	GlaxoSmithKline		Phase II	Ion-channel blocker/ GLU release inhibitor

*Outcome of first clinical trials in schizophrenia – pending.
GLU: Glutamate; GLY: Glycine; GLYT1: GLY type 1; NMDAR: NMDA receptor.

Table 2. Competitive environment (continued)

Compound	Company	Structure	Stage of development	Mechanism of action
Riluzole* (Rilutek®)	Aventis Pharma AG		Phase II	Ion-channel blocker/ GLU release inhibitor

*Outcome of first clinical trials in schizophrenia – pending.
GLU: Glutamate; GLY: Glycine; GLYT1: GLY type 1; NMDAR: NMDA receptor.

placebo, during a verbal cognitive challenge paradigm, demonstrate significant increase in temporal lobe activation that correlates with negative symptom amelioration [45].

A meta-analysis of the first 16 randomised, controlled trials with GLY, DSR and DCS (n = 343), obtained from the Cochrane Schizophrenia Group's Register of Trials, indicated that GLY and DSR, but not DCS, are effective in reducing negative symptoms of schizophrenia [46]. This analysis included diverse GLY site agonists doses and types of ongoing antipsychotic medications. Furthermore a characteristic pattern of therapeutic effects emerges from the outcome of studies performed so far in which identical GLY, DSR and DCS doses were used in conjunction with conventional neuroleptics, olanzapine and risperidone, or clozapine (Table 3). The most significant therapeutic benefits, covering not only negative, but also the cognitive and positive, symptom clusters, were achieved when the full agonists GLY and DSR were added to conventional neuroleptics. Similar, but less significant, effects were achieved when these compounds were used in conjunction with risperidone and/or olanzapine. Whether greater reductions occur during long-term treatment, or whether tolerance develops, is currently unknown. In some, but not all, studies, degree of negative symptoms improvement correlated significantly with baseline GLY levels, thus suggesting that patients with lowest pretreatment levels respond best to NMDA receptor agonist treatment [33].

In contrast, the addition of GLY and DSR to clozapine did not result in significant symptom changes, whereas the addition of DCS to treatment with this drug has actually resulted in a worsening of negative symptoms (Table 3). Because DCS functions as a GLY site agonist in the presence of low GLY concentrations, and as an antagonist in the presence of high concentrations [12,33], a likely explanation for the DCS-induced worsening of symptoms is that clozapine may already increase synaptic GLY levels. Recently, clozapine has been shown to block GLY and glutamine transport mediated by small neutral amino acid-like synaptosomal transporters (SNATs), providing a potential mechanism for both its unique therapeutic profile and the differential effects of NMDA receptor agonists in the presence of clozapine versus other antipsychotics [58].

Larger-scale, Phase II adjuvant treatment trials with GLY site agonists are presently in various stages of completion. A preliminary, total sample results analysis of a US National Institute of Mental Health (NIMH)-sponsored multi-centre study (CONSIST) comparing GLY and DCS effects did not indicate any significant therapeutic benefits of either GLY or DCS treatment [59]. A Stanley Foundation-sponsored multi-centre study (IMSER) performed in Israel is presently examining DSR adjuvant treatment effects in schizophrenia patients.

5.1.2 *Amino acid transport inhibitors*

Both GLY and DSR must be given at gram-level doses to significantly elevate CNS levels. An alternative approach to increase their CNS levels is the use of transport inhibitors, which raise their synaptic levels by preventing their removal from the synaptic cleft. Levels of synaptic GLY are tightly controlled by several transporters, including type I (GLYT1) and II (GLYT2) GLY transporters and system A-family SNAT transporters that serve to maintain low, subsaturating GLY concentrations in the immediate vicinity of the NMDA receptor complex [12,60]. GLYT1 and SNAT transporters predominate in the forebrain; GLYT1 transporters are closely associated with NMDA receptors, whereas GLYT2 transporters are co-localised with strychnine-sensitive inhibitory GLY receptors in the hindbrain. By increasing synaptic GLY-concentration in the vicinity of NMDA receptors, blockers of GLYT1 are expected to facilitate glutamatergic neurotransmission, and as such represent promising targets for pharmacological intervention against schizophrenia [12,60,61]. System A transporters are expressed in both neurons and glia, and transport a range of small neutral amino acids (e.g., serine, proline, glutamine) along with GLY. Blockade of these transporters, therefore, would also be expected to increase synaptic levels of amino acids relevant to glutamatergic function. Mechanisms underlying regulation of synaptic DSR levels in the brain are still poorly understood. Traditional transport systems show limited affinity for DSR, although selective high-affinity DSR transporters have recently been described. As with GLY transporters, these may represent selective targets for the modulation of brain DSR levels [14].

The use of GLY transport inhibitors (GTIs) for treatment of persistent symptoms of schizophrenia was first proposed in

Table 3. Patterns of symptoms change following addition of identical doses of GLY, DSR and DCS to conventional and atypical antipsychotic drugs for schizophrenia treatment.

Study	Treatment, daily dose	N	Study duration (weeks)	Symptom clusters outcome,%* (p)		
				Positive	*Negative*	*Cognitive*
Conventional antipyschotic drugs						
Heresco-Levy *et al.* (1999) [47]	GLY, 60 g	22‡	6	-20 (NS)	-39 (< 0.001)	-24 (0.01)
Tsai *et al.* (1998) [48]	DSR, 2.1g	29	6	-21.9 (0.004)	-20 (< 0.001)	-17.7 (0.004)
Goff *et al.* (1999) [49]	DCS, 50 mg	47	8	NS	-23 (< 0.02)	NS
Heresco-Levy *et al.* (2002) [50]	DCS, 50 mg	8‡	6	NS	-14 (< 0.05)	NS
Risperidone, olanzapine						
Heresco-Levy *et al.* (2004) [51]	GLY, 60 g	17‡	6	-11.4 (0.006)	-23 (< 0.0001)	-9.2 (0.02)
Heresco-Levy (2005) [52]	DSR, 2.1 g	23‡	6	-13 (0.001)	-16 (< 0.001)	-11.7 (0.001)
Evins *et al.* (2002) [53]	DCS, 50 mg	10	8	NS	-10 (0.02)	NS
Heresco-Levy *et al.* (2002) [50]	DCS, 50 mg	8‡	6	NS	-14 (0.05)	NS
Clozapine						
Evins *et al.* (2000) [54]	GLY, 60 g	27	8	NS	NS	NS
Diaz *et al.* (2001) [55]	GLY, 60 g	12	14	NS	NS	NS
Tsai *et al.* (1999) [56]	DSR, 2.1 g	20	6	-3.6 (NS)	-2.5 (NS)	-0.8 (NS)
Goff *et al.* (1999) [57]	DCS, 50 mg	17‡	13	NS	+13§ (< 0.005)	NS

*Mean percentage change in symptom scale scores; ‡Crossover study; §Positive value represents worsening of symptoms.
DCS: D-cycloserine; DSR: D-serine; GLY: Glycine; NS: Not significant.

1997 based on the behavioural actions of the GLY derivative glycyldodecylamide (GDA) [61]. GDA inhibits GLY transport at concentrations relevant to its behavioural actions, and subsequent studies using a series of GLY derivatives demonstrated that their potency in reversing PCP-induced hyperactivity correlated closely with potency in GLY transport inhibition [62]. Recently, several GLYT1 inhibitors – for example, *N*-(3-[4'-fluorophenyl]-3-[4'-phenylphenoxy]propyl) sarcosine (Allelix Neuroscience), ORG-24598 (Organon Laboratories) and SSR-504734 (Sanofi-Synthelabo) – have been reported to possess the preclinical profile of putative antipsychotics [60,61], but data for each compound are generally scant and none of these compounds has yet been developed clinically.

So far, the only clinical data concerning GTIs derives from two studies performed in Taiwan with the canonical *in vitro* inhibitor of GLYT1-mediated transport: GLY-derivative sarcosine (*N*-methylglycine) [104]. In a 6-week, controlled trial with chronic schizophrenia patients, sarcosine 2 g/day adjuvant treatment led to 17 ($p < 0.0001$), 14 ($p < 0.0001$) and 13% ($p < 0.0001$) reductions in positive, negative and cognitive symptoms, respectively, without inducing any significant side effects [63]. In a recently completed study [64] 65 risperidone-treated schizophrenia in-patients suffering from acute exacerbations were enrolled in a 6-week, randomised, double-blind trial comparing sarcosine 2 g/day, DSR 2 g/day and placebo. Patients who received sarcosine plus risperidone were reported to show significantly more symptoms improvement than the other two treatment groups. Cotreatment with DSR and risperidone did not differ significantly from risperidone monotherapy in this patient population.

Preclinical and clinical development of GLYT1 inhibitors is currently being pursued by major pharmaceutical companies [14,60,61]. In order to obtain increased efficacy and to lower required daily doses below the gram-level employed with sarcosine, lead GLYT1 inhibitors proposed for clinical testing show ~ 1000-fold greater potency than sarcosine at the GLYT1 transporters along with > 1000-fold selectivity versus other CNS targets [61]. The development of DSR reuptake inhibitors and alanine-serine-cysteine transporter 1 inhibitors [106] for the treatment of schizophrenia and other neuropsychiatric disorders is also being pursued.

5.2 AMPA receptor modulators

AMPA receptor modulators may provide an alternative strategy to NMDA receptor–GLY site stimulation for enhancing NMDA receptor function and facilitating glutamatergic

neurotransmission. GLU activation of AMPA receptors is thought to mediate fastest synaptic neurotransmission in the brain. AMPA receptors are composed of combinations of GluR1 – 4 subunits and work synchronically with NMDA receptor, providing the primary depolarisation necessary to unblock NMDA receptors and to permit calcium entry into the cell. Synergistically, Ca^{2+} entry through unblocked NMDA receptors triggers AMPA insertion into the postsynaptic density and synaptic strengthening [65].

Ligands bind to AMPA receptors by competing with GLU at the GLU binding site, or noncompetitively at other sites (allosteric modulators). Selective, high-potency AMPA antagonists have been developed and may be effective in conditions such as stroke or epilepsy, which are characterised by hyperglutamatergia. A distinct class of agents, developed to enhance glutamatergic function, are the AMPA-positive modulators termed AMPAkines [107], a family of compounds that act by increasing the peak and duration of GLU-induced AMPA receptor-gated inward currents [66]. AMPA receptor function facilitation by AMPAkines should enhance indirectly, in a use-dependent fashion, NMDA receptor-mediated long-term potentiation, a variant of synaptic plasticity widely regarded as a substrate of memory [32]. Overall, AMPAkines enhance glutamatergic activity in the cortex, stimulate memory-dependent processing in animal models and improve, acutely, memory capabilities in both young and aged humans without any apparent serious side effects [65,67-69]. Consequently, AMPAkines are under development at present for treatment of cognitive dysfunction in various neuropsychiatric disorders.

As AMPA receptors function in concert with NMDA receptors, AMPAkines have also been proposed as potential therapeutic agents for schizophrenia. When added to low doses of clozapine, or conventional antipsychotics, AMPAkines synergistically block methamphetamine-induced rearing behaviour, which is an effect believed to predict antipsychotic efficacy [70]. Moreover, AMPAkines may induce DSR release into synapses by glia in response to their stimulation of AMPA receptors [71], thus contributing to increased NMDA receptor function and enhanced therapeutic effects.

1-(quinoxalin-6-ylcarbonyl) piperidine (CX-516, Cortex Pharmaceuticals, Organon) is the first AMPAkine to reach Phase I trials in schizophrenia. So far, the results of a pilot, double-blind trial in which patients taking clozapine were randomised to receive either CX-516, ≤ 900 mg t.i.d., or placebo for 4 weeks have been published [68]. Eighteen schizophrenia patients participated in this study and significant CX-516-induced improvements in cognitive and negative symptoms were reported. The only possible treatment-related side effect was hypertension, which was seen in one patient. CX-516 ≤ 1200 mg t.i.d. has also been assessed with no clear benefit in a small study (n = 8) as monotherapy for schizophrenia patients partially refractory to treatment with traditional neuroleptics [69]. At present, a more powerful compound with improved pharmacological properties (CX-717, Cortex Pharmaceuticals, Organon) is examined as adjuvant treatment for schizophrenia in a larger-scale study and additional, structurally distinct, AMPA receptor modulators are being developed by various research groups [67]. Furthermore, the NIMH-sponsored network entitled Treatment Units for Research on Neurocognition in Schizophrenia (TURNS) has recently selected the AMPAkine CX-619/ORG-24448 (Cortex Pharmaceuticals, Organon) as one of the first compounds to undergo testing as part of its efforts to facilitate the development of medications for enhancing neurocognition. The TURNS programme represents an innovative initiative that aims to identify compounds of interest and conduct proof-of-concept clinical studies on the treatment of cognitive deficits in schizophrenia [201].

5.3 mGluR modulators

mGluRs are G protein-coupled receptors that are divided into three groups and include eight subtypes termed mGluR1 – 8. Group I receptors function predominantly to potentiate both presynaptic GLU release and postsynaptic NMDA neurotransmission, with mGluR5 receptors showing significant colocalisation with NMDA receptors in rodents. Group II and III receptors, in general, serve to limit GLU release, particularly during conditions of GLU excess. Thus, group I agonists or positive modulators would be expected to stimulate NMDA receptor-mediated neurotransmission, and group I antagonists to inhibit it. In contrast, group II/III agonists or positive modulators would be expected to inhibit presynaptic GLU release [10,12,72]. Development of mGluRs modulators as therapeutic targets in schizophrenia is thus based on two alternative conceptualisations of the disorder. Effectiveness of group I agonists is predicted based on the models that postulate low NMDA receptor activity and/or GLU levels as being pathophysiological in schizophrenia, whereas use of group II/III agonists would follow models that postulate that GLU hyperactivity may be pathophysiological.

Preclinical studies have assessed the ability of group I (mGluR1, mGluR5) agonists to reverse effects induced by amphetamine, PCP and other psychotomimetics. The mGluR5 agonist 2-chloro-5-hydroxyphenylglycine has been found to reverse prepulse inhibition-disruptive effects of amphetamine in rodents. Similarly, both nonselective and group I-selective agonists inhibit PCP-induced DA release in rodent prefrontal cortex [10,12]. Several high-affinity agonists have been developed, including (-)-2-oxa-4-aminobicyclo[3.1.0.]hexane-4,6-dicarboxylate (LY-379268) and the related compound LY-354740 (Eli Lilly), that permit characterisation of the effects of group II agonists in both preclinical and clinical studies. An initial study with LY-379268 demonstrated its ability to block PCP-induced increases in prefrontal GLU, along with PCP-induced impairments in working memory. Similarly, LY-3279268 has been shown by a variety of groups to inhibit PCP-induced hyperactivity during both acute and repeated administration, and reverse PCP-induced behaviours in monoamine-depleted mice [72]. A recent study

also suggests that LY-354740 may reduce working memory impairments and perhaps psychotic symptoms transiently produced by ketamine in healthy human subjects [10].

Based on the effect of group II agonists on prefrontal glutamatergic hyperactivity, it has been proposed that these agents may be therapeutically beneficial in treating persistent cognitive deficits in schizophrenia [72,73]. At present, however, the degree to which psychotomimetic effects of PCP are related to alterations in glutamatergic versus dopaminergic neurotransmission is not known [12]. Clinical trials with mGluR2 agonists may thus also help clarify pathophysiological mechanisms in schizophrenia.

Overall, in comparison with NMDA receptor-based approaches, mGluRs modulators are presently in relatively early stages of development as potential drugs for schizophrenia. Primate studies and clinical trials are warranted in order to validate this molecular target for the treatment of this illness.

5.4 Ion-channel blockers/GLU release inhibitors

Recently, two compounds, lamotrigine and riluzole, which are widely used for the treatment of epilepsy and amyotropic lateral sclerosis (ALS), respectively, have been proposed and are presently evaluated for schizophrenia treatment, mainly on the basis of their ability to inhibit excessive presynaptic GLU release.

5.4.1 *Lamotrigine*

Despite limited supporting data for this application, anticonvulsant agents are widely prescribed to enhance the efficacy of antipsychotic drugs in schizophrenia [74]. Lamotrigine (3,5-diamino-6-[2,3-dichlorophenyl]-1,2,4-triazine) is a novel antiepileptic drug that is chemically unrelated to any currently available anticonvulsant medication. It has been extensively evaluated and is commercially available for the treatment of epilepsy [108]. Furthermore, lamotrigine is effective in the treatment of affective disorders [75] and, in addition to its mood-stabilising properties, significant quality of life and cognitive function improvements were reported with this drug in epilepsy [76] and Alzheimer's disease [77].

Lamotrigine reduces GLU release via blockade of voltage-dependent ion channels, particularly sodium channels and P- and N-type calcium channels and an outward potassium channel [78]. Thus, it was hypothesised that lamotrigine would attenuate those effects induced by NMDA receptor noncompetitive antagonists that are mediated by disinhibition of GLU release [10]. Consistent with this hypothesis, it was found that lamotrigine pretreatment reduces ketamine-induced psychosis, negative symptoms, and dissociation-like perceptual alterations, and increases the euphoric or stimulatory effects of ketamine in healthy humans [79]. Furthermore, lamotrigine showed efficacy in two animal models that may have predictive therapeutic value in schizophrenia: NMDA receptor antagonist-induced neurotoxicity [36] and NMDA receptor antagonist disruption of prepulse inhibition of the startle response [80].

So far, clinical trials with lamotrigine in schizophrenia have focused on the augmentation of clozapine treatment in refractory patients. In three open-label studies [81-83] lamotrigine adjuvant treatment (100 – 400 mg/day) produced significant (≤ 75%) symptom reductions in clozapine-maintained, treatment-resistant patients. In a randomised, controlled, 14-week study performed with 34 male forensic schizophrenia inpatients resistant to conventional neuroleptics and clozapine treatment, the addition of lamotrigine ≤ 200 mg/day to clozapine regimens significantly improved positive and general psychopathology symptoms [84]. Recently, the results of the first controlled trial in which fixed dose lamotrigine 400 mg/day was added to ongoing treatment with conventional or atypical antipsychotics have been published [85]. The completers ($n = 32$) analysis in this pilot study indicated, following 10 weeks of lamotrigine treatment, significant mean reductions in positive (-42%, $p < 0.03$) and general psychopathology (-36%, $p < 0.03$) symptoms, whereas the negative symptoms cluster was not significantly affected. Larger-scale investigations of lamotrigine adjuvant treatment in schizophrenia are at present ongoing.

5.4.2 *Riluzole*

Riluzole is approved by the US FDA as a neuroprotective agent for use in ALS and, similarly to lamotrigine, inhibits voltage-dependent Na^+ channels, thus resulting in decreased GLU release [86,109]. It was the first medication to show some impact on survival for ALS patients and, due to its pharmacological characteristics, has been proposed for evaluation as possible treatment in a number of psychiatric disorders, including schizophrenia [109] and affective disorders [87]. Recent case studies reported beneficial riluzole effects in depression and obsessive-compulsive disorder [87,88]. Clinical trials are warranted in order to assess riluzole treatment effects in schizophrenia.

6. Development issues

On the basis of research done so far, a number of challenges may be predicted in the process of development of glutamatergic neurotransmission modulators for use in schizophrenia treatment. Main unresolved issues presently include the estimation and establishment of: i) primary symptom targets; ii) potential effects on cognition; iii) optimal adjuvant treatment regimes; iv) optimal dose ranges; v) characteristic side effects profiles; and vi) therapeutic effects for different types of patients and illness stages.

Different types of glutamatergic modulators may differ in terms of their characteristic therapeutic effects profiles. NMDA receptor-GLY site agonists (Table 3) and AMPAkines [68] may represent the prototypes for medications affecting mainly the negative symptoms and cognitive deficits domains. Therapeutic effects of GLY site agonists have also been reported against antipsychotic drug-induced EPS and tardive dyskinesia [51,52]. However, because no monotherapy trials with these agents have

yet been performed, it is not clear whether the relatively modest effects of GLY and DSR registered against positive symptoms reflect pharmacological limitations of this type of compounds, or a ceiling effect resulting from their addition to ongoing established antipsychotics. Clarification of this issue would further guide the hypothesised use of NMDA receptor modulators. On the other hand, trials performed so far with the GLU release inhibitor lamotrigine suggest a main effect of this type of compound on positive symptoms and general psychopathology, whereas negative symptoms were not significantly affected [84,85]. These preliminary findings require larger-scale replication in studies using well-defined outcome criteria. Furthermore, at both conceptual and practical levels they suggest the intriguing possibility of assessing combined glutamatergic treatments (e.g., GLY site agonist plus GLU release inhibitor) in schizophrenia treatment.

In general, the first-generation small clinical trials with glutamatergic modulators have assessed cognitive parameters on the basis of symptom scales (e.g., Positive and Negative Syndrome Scale) ratings. Although the results of some of these studies may be viewed as encouraging, cognitive symptom subscales are only poorly related to performance-based measures of cognitive capabilities. Consequently, comprehensive neurocognitive testing-based assessments of the effects of glutamatergic modulators on cognitive functions are warranted. State-of-the-art neurocognitive tests batteries are presently employed in ongoing multi-centre studies (e.g., IMSER, TURNS) and should be regarded as standard procedures in future studies in this field.

Glutamatergic agents may not work equally well in combination with all antipsychotic medications. In regard to GLY site agonists, the most significant therapeutic effects were registered when used in conjunction with conventional neuroleptics, whereas clozapine appears to stand apart from all antipsychotics (Table 3). Full GLY site agonists appear to be ineffective when used as adjuvants to clozapine [54-56], whereas partial agonists may even exacerbate symptoms [57]. In contrast, the prototypical AMPAkine CX-516 has been reported to be effective in combination with clozapine [68] and lamotrigine appears to be effective when prescribed in combination with this drug, whereas it may work less well in combination with other antipsychotics [83]. A better understanding of the mechanisms of action that account for the diverse outcomes of glutamatergic modulators/clozapine treatment regimens may help explain the unique therapeutic profile of clozapine and contribute to the development of glutamatergic treatment strategies in schizophrenia.

The optimal dose ranges at which glutamatergic agents may be used in schizophrenia are presently unknown. For example, GLY, DSR and sarcosine doses of > 60, 2.1 and 2 g/day, respectively, although potentially beneficial, have not yet been assessed. Lamotrigine doses of ≥ 200 mg/day were found to be effective in some studies [84,85], but were also reported as detrimental in comparison with lower dosages [10]. Dosage issues require further research and are related to the establishment of the side effects profiles of glutamatergic modulators. Overall, these drugs seem to be practically devoid of the characteristic motor and metabolic side effects occasionally encountered with conventional and atypical antipsychotics. However, further studies are needed in order to consolidate these observations and monitor specific areas of concern. Although significant clinical or laboratory-determined side effects, including DSR kidney function effects [52], have not yet been reported with GLY site agonists, and GLY and DSR have been reported to actually decrease EPS severity [51,52], additional studies are clearly required in order to address long-term safety issues connected with the administration of this type of compound. Concerning lamotrigine, benign and serious rash are known side effects. Calabrese *et al.* [89], after reviewing the prospectively collected data from double-blind studies of lamotrigine in the treatment of mood disorders (n = 1198), reported that the risk of serious rash was nil. The rate of benign rash was determined to be 8%. This is on par with lamotrigine postmarketing experience, which has shown that the risk of rash is essentially limited to the first 8 – 12 weeks of treatment and can be limited by adhering strictly to the currently recommended slow initial rate of dose titration. Although the data furnished by Calabrese *et al.* [89] is encouraging, the prescribing information for lamotrigine nonetheless reports an overall 3% risk of Steven–Johnson syndrome in adults [90] and the occurrence of both benign and serious forms of rash should be carefully monitored in future studies involving schizophrenia patients.

Until now, glutamatergic treatments have generally been assessed mainly in chronic patients characterised by long illness duration, prominence of negative symptoms and paucity of positive symptoms and EPS. Further studies are needed in order to explore the possible benefits of glutamatergic modulators for patients with different symptom profiles and for patients in which predictors of treatment response (e.g., low GLY serum levels), as suggested in previous studies, may be evidenced. An additional and potentially promising domain in which the use of glutamatergic, especially NMDA receptor, modulators should be assessed, involves secondary prevention of disease (i.e., tackling of schizophrenia manifestations at a prodromal or relatively early stage of illness).

Ultimately, as highlighted by Krystal *et al.* [10], an additional challenge facing the development of glutamatergic compounds for schizophrenia may be economic and regulatory. Overcoming these inherent difficulties would require the willingness of the pharmaceutical industry to explore a path of high financial risk, involving the development of schizophrenia medications that are characterised by molecular mechanisms not explored so far and that may not be sufficient as stand-alone pharmacotherapies.

7. Expert opinion

Despite major advances in antipsychotic medications, there is clearly a need for innovative treatment strategies in schizophrenia that will ensure increased effectiveness against negative symptoms and cognitive dysfunction, ultimately

leading to disease outcome modification. Within this framework, mainly from a conceptual perspective at present, modulators of glutamatergic neurotransmission seem to have the potential for becoming clinically useful compounds to be used in daily practice.

The neurotransmitter role of GLU was definitively established only in the 1970s and GLU receptors were not differentiated until the 1980s. Consequently, drug development focusing on glutamatergic neurotransmission is historically and substantially behind that for other neurotransmitter systems. Nevertheless, two basic considerations are encouraging in the context of this complex endeavour. First, available data suggest, mainly for NMDA receptor agonists with which most clinical research has been done, that glutamatergic modulators may represent a distinct, new class of medications. This hypothesis is based on the fact that these types of compounds differ from both conventional and presently available atypical antipsychotics not only in terms of their mechanisms of action, but also in terms of their characteristic therapeutic and side-effect profiles. Furthermore, if glutamatergic modulators will eventually be established as effective against negative and/or cognitive symptoms in schizophrenia, it is reasonable to predict that, similarly to antipsychotics, their use will not be limited to this illness, but will encompass a variety of disorders in which these or similar symptoms clusters play a prominent role. For example, the usefulness of glutamatergic treatments has already been proposed and/or is being assessed in neurodegenerative disorders, autism, post-traumatic stress disorder and depression.

Specifically for the NMDA receptor, the GLY regulatory site is a promising target, with GLY site agonists currently in active development as schizophrenia treatments. However, due to intertwined efficacy and pharmacokinetic limitations, it is unlikely that GLY or DCS will become widespread treatments. DSR, if proven to be safe and significantly effective in future studies, has the potential to be recommended at least as add-on pharmacotherapy in schizophrenia. The advantages of this compound include the relative low dose requirements and the fact that it probably represents the natural neurotransmitter acting at the GLY site with no other known site of action throughout the nervous system. Furthermore, as with monoamine and acetylcholine systems, synthetic and degratory enzymes and especially neurotransmitter reuptake systems (e.g., by use of GLYT1 inhibitors), may prove highly effective psychopharmalogical development targets. More recently described DSR and SNAT transporters may serve as additional targets. It may ultimately be possible to develop ligands which, by virtue of contrasting affinities and/or efficacies, preferentially modulate specific populations of GLY site/NMDA receptor subtypes that may be differentially involved in schizophrenia.

Positive allosteric AMPA receptor modulators have been developed and are undergoing clinical development in schizophrenia (mainly for cognitive dysfunction) and other neuropsychiatric disorders. Direct agonists, antagonists and allosteric modulators have been developed for group I and II mGluRs and are undergoing clinical development for both schizophrenia and anxiety disorders. As with NMDA receptor modulators, many of the key compounds required for both preclinical and clinical testing in these areas remain proprietary. As these compounds become more generally available, it is expected that progress in development of glutamatergic therapies will further accelerate.

Preliminary clinical evidence suggests that the novel anticonvulsant lamotrigine, which inhibits excessive GLU release, may augment antipsychotic efficacy in some patients diagnosed with schizophrenia. However, replication of these findings is required before the possible inclusion of lamotrigine, along with carbamezepine and valproate, among the antiepileptic drugs used as add-on therapy in schizophrenia. In the case of riluzole, clinical data are pending.

Hypothetically, due to their therapeutic profile and to the role of GLU in brain physiology and development, glutamatergic modulators may, if proven effective, enrich the scope of pharmacological treatment in schizophrenia. New areas of investigation likely to be explored during the next years would include their use in maintenance treatment, with resulting limitation of exposure to antipsychotic drugs side effects and/or as early or 'preventive' treatments to be assessed during prodromal stages and/or following first psychotic episode in schizophrenia.

It should be stressed that no glutamatergic modulator has yet reached the market, and conclusive experimental and clinical research is still needed to more fully appreciate the potential role of these types of compound in schizophrenia management. Nevertheless, although the full range of benefits and limitations of these compounds remains to be demonstrated, glutamatergic treatments presently represent one of the most hopeful avenues for development of innovative pharmacological strategies in schizophrenia.

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Review

Glutamate and anxiety

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Abstract

Although glutamate is a simple molecule, its actions in the limbic system and areas concerning anxiety are complex and widespread. These actions are mediated through different combinations of ionotropic and metabotropic glutamate receptors. Preclinical studies have shown that compounds active at NMDA, AMPA/kaïnate and metabotropic receptors might have anxiolytic properties. The major research effort so far has been directed towards the development of compounds which modulate the function of NMDA receptors. In general, the utility of NMDA and AMPA/kaïnate antagonists is greatly hampered by adverse effects. For the treatment of clinical anxiety disorder a more delicate regulation of the glutaminergic system is required. It is encouraging that different ways to fine-tune the glutaminergic system are emerging, e.g., modulators of the glycine site and compounds acting at the AMPA receptor. Metabotropic glutamate receptor agonists and antagonists are in particular promising in this respect. It can be expected that selective modulators of glutamate activity will be of great clinical significance for the treatment of anxiety disorders.

Keywords: Anxiety; Glutamate; Fear conditioning; Animal models; Therapeutic

1. Introduction

The neurobiological underpinnings of anxiety have recently received much attention. These studies have produced findings that are important to generate hypotheses about the biology of anxiety. Several neurotransmitters have been implicated in the genesis of anxiety with earlier data highlighting the γ-aminobutyric acid (GABA) system, the locus of action of the benzodiazepines. Apart from the GABA system, other neurotransmitters such as serotonin (5-hydroxytryptamine; 5-HT) and noradrenaline (NE) have been implicated in anxiety disorders. Several neuropeptides such as adrenocorticotropic hormone (ACTH), corticotropic releasing hormone (CRH), neuropeptide-Y (NPY) and cholecystokinin (CCK) also seem to play a role in the pathogenesis of anxiety.

Recent developments in the neurobiology of anxiety have highlighted the neurotransmitter glutamate as an important element in anxiety and anxious behaviour. In most synapses the actions of the inhibitory neurotransmitter GABA are opposed by the effect of glutamate, which is the major excitatory neurotransmitter in the central nervous system in mammals. This review will examine pathophysiological and therapeutic hypotheses of glutamate, its receptors and anxiety.

2. Neurobiology of anxiety

From an evolutionary point of view it may be postulated that the anatomic core of fear/anxiety is represented by a set of interrelated limbic structures: the septo-hippocampal system, certain nuclei of the amygdaloid complex and areas of the hypothalamus, as well as the periaqueductal grey matter of the midbrain (Charney et al., 1996). Their basic functions are to evaluate the extent to which situations are threatening for the individual and to select appropriate responses in order to generate adequate patterns of defence. The hippocampus is thought to play a role in processing of context-related information and the expression of anxiety responses to environmental signals. The periaqueductal grey matter, in addition to its role in endogenous pain suppression, is the caudal pole of a longitudinal organised neural system which modulates fear and anxiety. Although many limbic as well as cortical areas are involved in the behavioural expression of anxiety, the amygdaloid complex is

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thought to play a crucial role (for review see Davidson, 2002; Davis, 1997). A large and consistent literature indicates that electrical activation of the amygdala produces a pattern of behavioural changes in animals that closely resembles a response after a fearful stimulus. In contrast, lesions of the amygdala block reactions to fearful stimuli (Maren, 1996). The amygdala and its many efferent projections represent a central fear system involved in the acquisition, consolidation and expression of conditioned fear (Walker and Davis, 2002). Most of our knowledge about the biological underpinnings of anxiety in the amygdala and limbic circuit has been studied using fear conditioning. Relevant background information about fear conditioning has been summarised by LeDoux (1998). In classical fear conditioning a neutral stimulus, which has little behavioural effect by itself, is consistently paired with a strong aversive stimulus. Following a small number of pairings, the neutral stimulus produces effects formerly only produced by the strong aversive stimulus. This change is not seen when the stimulus is presented in an unpaired fashion (Davis et al., 1994; LeDoux, 1994). A cellular analogue to this classical conditioning can also be made. When a weak input to a postsynaptic cell is paired with activation by a second, stronger signal to the same cell, a small number of pairings will be sufficient to increase the synaptic strengths, resulting in enhancement of the synaptic transmission. Details about these synaptic transmissions in the amygdala have provided important information regarding fear conditioning (Rogan et al., 1997).

Glutamate and GABA are abundant in the amygdala and other limbic and cortical structures. In the treatment and neurobiology of anxiety disorders some interest has been focussed on possible abnormalities in GABA neurotransmission and the benzodiazepine receptor (Haefely, 1990; Ninan et al., 1982). However, thus far attention has been focussed mainly on the role of serotonin (Jones and Blackburn, 2002). In the last decades, controlled clinical studies have demonstrated the therapeutic efficacy of drugs selectively affecting 5-HT receptors in different anxiety disorders. These advances have kindled interest in the role of 5-HT in anxiety resulting in a wealth of data on the morphological and functional aspects of the 5-HT neuronal systems. Early models attributed an anxiogenic function to endogenous 5-HT. Paradoxically, empirical data have provided results pointing to an anxiogenic as well as an anxiolytic role of 5-HT. This dual role assumes two independent 5HT systems performing different behavioural functions. Graeff (1993) hypothesised that 5-HT possibly facilitates defensive behaviour by acting on the amygdala while simultaneously inhibiting active defensive patterns organised in the central grey matter and periphery. Viewed in this way, threatening stimuli could activate brain defence mechanisms and 5-HT systems independently. Consequently, 5-HT is not responsible for defence mechanisms per se but only modulates them. This view assumes an indirect influence of serotonin on anxiety. Several other neurotransmitters and neuropeptides play a role in the complex neuroanatomical pathways in anxiety and fear conditioning (for review see Ninan, 1999). Emerging is the importance of corticotropin-releasing factor secreting neurones in the central amygdala nucleus. CRH is hypothesized to facilitate anxiety reactions by activation of this central nucleus (Shepard et al., 2000). Anxiogenic proportions have been ascribed to other neuropeptides as well, for example cholecystokinin.

The noradrenergic and dopaminergic systems are believed to increase arousal in response to threat. The noradrenergic locus coeruleus stimulate the periaqueductal grey via an indirect pathway through the amygdala (Charney et al., 1996).

3. The glutamate neuronal system

Until 1970 it was not recognised that excitatory amino acids such as glutamate might play a physiological role in brain functioning. Since then it has been discovered that the glutamate system can be found in all parts of the human brain (Collingridge and Lester, 1989). This ubiquitous nature of glutamate excitation supports a role for glutaminergic neurotransmission in several cerebral functions (Danysz et al., 1995). For instance, the glutamate system is known to play an important role in cognition, learning and memory (Davis et al., 1994; Maren, 1996; LeDoux, 1994), the neural plasticity of synaptic connections (Kaczmarek et al., 1997), pain perception (Klepstad et al., 1990) and in the regulation of neuroendocrine secretion (Brann and Mahesh, 1994). The spectrum of excitation by glutamate ranges from normal neurotransmission, to excess neurotransmission causing pathological symptoms such as mania or panic, to excitotoxicity resulting in minor damage to dendrites. Hyperactivity of the glutamate system is associated with several neurodegenerative diseases, e.g., Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis and schizophrenia (Danysz et al., 1995). The glutamate system might impact progressive neurodegeneration by an excitotoxic mechanism. Slow progressive excitotoxicity can be associated with degeneration such as seen in Alzheimer's disease. Sudden and catastrophic excitotoxicity can cause neurodegeneration as in stroke (Cotman et al., 1995).

3.1. Glutamate receptors

For years the central effects of glutamate were thought to be exclusively mediated by ion channel mechanisms. However, glutamate receptors can now be categorised into two major groups, (I) ionotropic and (II) metabotropic receptors. This categorisation is based on intracellular/extracellular coupling and on different pharmacological and biochemical characteristics. Ionotropic receptors can be subdivided into

N-methyl-D-aspartate (NMDA), kainate and quisqualate receptors named after the agonists that selectively bind to these receptors. These synthetic selective agonists resemble either glutamate or aspartate. The quisqualate receptor has been renamed as the amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor (Hollmann and Heinemann, 1994; Nakanishi, 1992).

The NMDA receptor-channel complex has several characteristic features. There are several regulatory sites on this NMDA receptor complex. Three of these modulatory sites are outside the ion channel (the neurotransmitter glycine site, the polyamine site and the zinc site) and are excitatory in nature. The inhibitory modulator sites are located inside the ion channel. Magnesium ions can block the calcium channel at one of these sites. The other inhibitory site is sometimes called the 'PCP site' because the psychomimetic agent phencyclidine also binds to this site.

Precise modulation is required for normal neuronal functioning, depolarisation of the NMDA receptor results in a slow rising, long lasting current (Cotman et al., 1995).

In most CNS synapses, NMDA receptors coexist with either AMPA or kainate receptors. These latter receptors are thought to be involved in amplification of the glutamate signal. The level of concurrent depolarisation depends on AMPA/kainate activation and other modulator signals. Both AMPA and kainate receptors mediate fast excitatory synaptic transmission (Cotman et al., 1995).

The ionotropic glutamate receptors are distributed throughout the brain. However, different types of receptors exhibit regional and functional variability. Overall the density of NMDA receptors is high in cortical and limbic regions. The distribution of AMPA and kainate receptors is similar to that exhibited by the NMDA receptor, consistent with their common action as a functional pair. The cortical and limbic localisation of these receptors accounts for its effects on cognition, perception and mood (Krystal et al., 1999).

In the 1980s it became apparent that glutamate also acts on a class of non-ionotropic receptors or G-protein bound receptors also termed the 'metabotropic' receptor (Pin and Duvoisin, 1995). These glutamate receptor subtypes are often on the same neurones and in almost all cases interact within neural networks. To date eight metabotropic glutamate receptors (mGluRs) have been cloned. These receptors are present at both presynaptic and postsynaptic sites (Fig. 1). The eight mGluRs are subdivided into three groups, each possessing similar pharmacology and second messenger coupling. Metabotropic glutamate receptor subtypes are also differentially distributed within the CNS. Studies in rats have shown that Group II mGluRs are highly localised in the forebrain regions and limbic structures (Ohishi et al., 1993). This expression of mGluRs in different regions provides a way to fine-tune glutaminergic neuronal transmission within specific synapses (Schoepp and Conn, 1993).

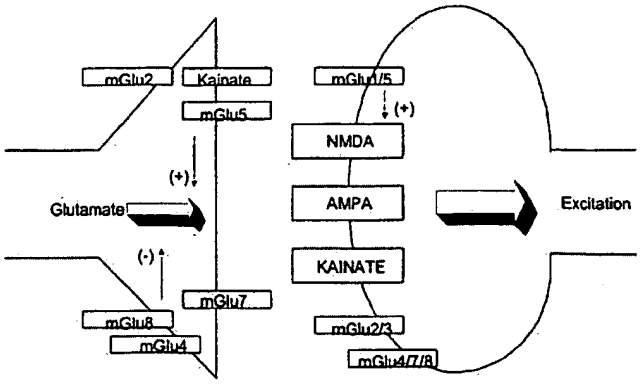

Fig. 1. Glutamate receptor subtypes.

In short, the AMPA and kainate receptors evoke fast synaptic responses and in turn promote the activation of voltage-dependent NMDA receptors. The mGluR subtypes exert long-lasting actions through the activation and inhibition of intracellular signals. Coherent cortical function depends on a balanced action of glutamate receptors of different classes.

4. Glutamate and anxiety

The glutaminergic system is thought to play a major role in the pathogenesis of anxiety and fear conditioning.

As mentioned before, treatments that improve the excitability of output neurones in the basolateral amygdala improve aversive conditioning. Alternatively, treatments that decrease the excitability of these neurones produce anxiolytic effects (LeDoux, 1994, Maren, 1996). Decrease in excitatory output in the amygdala can be achieved by decreasing the excitatory glutaminergic transmission. Blocking the basal glutamate excitation generated by ionotropic receptors could elicit a significant anxiolytic effect. Indeed, the administration of antagonists of the NMDA and non-NMDA type receptors into the basolateral amygdala has been shown to reduce anxiety in animal models (Kim and McGaugh, 1992; Miserandino et al., 1990). An alternative way to decrease excitatory output in the amygdala could be achieved by an increase in GABA neurotransmission. The anxiolytic benzodiazepines increase GABA neurotransmission and induce a decrease in excitatory output of the amygdala. There appears to be a balance between GABA receptor mediated inhibition and glutamate receptor mediated excitation that regulates behavioural and physiological responses associated with anxiety (Sajdyk and Shekhar, 1997). In addition, there is mounting evidence, gathered from various parts of the CNS that both inhibitory (GABAergic) and excitatory (glutaminergic) transmission can be modulated by presynaptic excitatory amino acid receptors (Salt and Eaton, 1995). It is likely that these receptors are of the mGluR type(s). Presumably this presynaptic inhi-

bition is achieved through activation of a metabotropic glutaminergic autoreceptor. Thus, GABA receptors produce inhibitory actions in the amygdala. In contrast, glutamate receptors can produce both excitatory and inhibitory actions in the amygdala and the degree of ionotropic and metabotropic activation is likely to be an important determinant of amygdaloid cell excitability (Maren, 1996). These anxiogenic or anxiolytic actions of different glutamate receptors in the amygdaloid cells can be better understood in relation to fear conditioning. Details about the synaptic transmission in the amygdala has been studied using a cellular analogue to fear conditioning (Rogan et al., 1997). In classical fear conditioning, a neutral stimulus elicits release of glutamate onto neurones in the amygdala. This glutamate binds to both NMDA and AMPA/kaïnate receptors. However, this might not produce much of a behavioural response. Only weak activation of AMPA/kaïnate receptors occurs and in addition, the NMDA-channel is not permeable by a partial blockade of Mg^{2+}. However, presentation of a strong aversive stimulus at about the same time can further depolarise the neurone and relieve the Mg^{2+} blockade leading to a behavioural response. This triggers events that increase the ability of the previously neutral stimulus to activate the neurone, enabling it to produce effects similar to those previously produced only by the aversive stimulus (Davis et al., 1994).

NMDA antagonists can prevent this process (Bliss and Collingridge, 1993). The role of NMDA antagonist in fear conditioning based on intra-amygdala injection of NMDA antagonists had been studied by Miserandino et al. (1990) who found that intra-amygdala blockade of NMDA receptor function disrupts acquisition of fear conditioning. However, the application of NMDA alone is usually not sufficient to induce a change. Other stimuli, in addition to NMDA receptor activation, may be required to facilitate the process of fear conditioning. The role of AMPA receptors and metabotropic glutamate receptors was also analysed in this respect (Walker and Davis, 2002). For extensive information on the role of metabotropic receptors in fear conditioning the reader is referred to the article of Watkins and Collingridge (1994).

5. Glutamate in animal models of anxiety

Pharmacological agents, that block glutamate output, may be of therapeutic use for the treatment of anxiety. Glutamate receptor ligands are effective in animal models for anxiety by preventing fear conditioning and by having direct anxiolytic effects. Glutamate agonists and antagonists have been tested in different anxiety paradigms. Generally, two main categories of animal models can be distinguished, conditioned behaviour models and unconditioned behaviour models. Conditioned behaviour models use conflict tests. Examples of unconditioned behaviour models are the social interaction paradigm, the elevated plus maze, the ultra sonic vocalisation paradigm and the acoustic startle paradigm. The glutamate receptor ligands used in several anxiety models are shown in Tables 1–3.

Most studies have focussed on the role of NMDA glutamate receptors (Table 1). There are at least four sites at which antagonists can block the activation of the NMDA complex. Competitive antagonists block the NMDA site itself, non-competitive antagonists like phencyclidine act by blocking the cation channel. Inhibition of NMDA receptor activity could also be achieved via blockade of NMDA/glycine-sensitive sites. Many studies show functional differences between antagonists acting at the different sites associated with the NMDA receptor complex (Table 1), even compounds with similar potencies under in vitro conditions can have different functional profiles in vivo.

Overall, the effects of phencyclidine-like NMDA receptor antagonists are not unequivocal in nature and appear to be less specific as compared to the effects of competitive NMDA receptor antagonists (Wiley, 1997). Consequently, the search for 'NMDA' compounds with anxiolytic properties was focussed on the development of competitive NMDA channel blockers and glycine receptor antagonists (Wiley et al., 1995; Dunn et al., 1989; Bennet and Amrick, 1986).

A limited number of investigations were carried out with non-NMDA receptors ligands (Table 2 and 3) since active and selective ligands for these non-NMDA receptors were only available to a limited extent. AMPA antagonists displayed anxiogenic actions in three studies with conditioned and unconditioned tests (Benvenga et al., 1993; Karcz et al., 1995; Kotlinska and Liljequist, 1998a,b).

Behavioural studies of mGLuR activation or inhibition are also scarce.

The heterogeneous family of metabotropic receptors has only recently been cloned and the discovery of compounds that selectively modulate the receptor is still in its infancy (Schoepp and Conn, 1993). In the last 10 years different groups (i.e., I, II and III) of metabotropic receptors agonists and antagonists have been developed. Although interesting in vitro, most compounds are not yet useful in vivo because of poor bioavailability and low potency. Recently, systemically active mGlu5 receptor antagonists were discovered and one derivative demonstrated anxiolytic potential (Brodkin et al., 2002; Pilc et al., 2002; Spooren et al., 2000; Tatarczynska et al., 2001). A high expression of mGlu5 receptors in the limbic forebrain regions was observed. So far, one metabotropic receptor agonist showed potent central effects when tested systemically (Helton et al., 1997, Klodzinska et al., 1999; Shekhar and Keim, 2000). This compound acts at mGlu2 and mGlu3 receptors, distributed mainly in the limbic system.

Table 1
NMDA receptor antagonists

Substance	Anxiety test	Anxiety	Motor	Authors
Non competitive antagonists				
Ketamine	CT, SI, X-maze	=, ↑		Silvestre et al. (1997)
MK801	CT	↓ =	=, ↑	Xie and Commissaris (1992), Corbett and Dunn (1991), Koek and Colpaert (1991), Jessa et al. (1996)
	SI	↓	=	Corbett and Dunn (1991), Dunn et al. (1989)
	X-maze	↓	=	Corbett and Dunn (1991), Dunn et al. (1989), Fraser et al. (1996)
		=	↑	Criswel et al. (1994)
PCP	Open field		↑	Kotlinska and Liljequist (1998), Plaznik et al. (1994), Jessa et al. (1996)
	USV	↓	↓	Vry De et al. (1993), Kehne et al. (1991)
	CT	↓, =		Porter et al. (1989), Sanger and Jackson (1989)
	X-maze	=		Wiley et al. (1995)
Mem, am	USV	↓		Vry De et al. (1993)
	CT, X-maze	=		Karcz et al. (1997)
Competitive antagonists				
NPC 17742	X-maze, CT	↓	=	Wiley et al. (1995), Willetts et al. (1994)
CPP	CT	↓		Corbett and Dunn (1991), Koek and Colpaert (1991)
CGS 19755	CT	↓		Bennet and Amrick (1986), Koek and Colpaert (1991)
AP5	X-maze, SI, USV	↓		Dunn et al. (1989), Kehne et al. (1991)
AP7	ASP, CT, X-maze, SI			Anthony and Nevins (1993), Bennet and Amrick (1986), Stephens et al. (1986) Dunn et al. (1989), Plaznik et al. (1994)
CGP 37849	Open field, CT	↓	=, ↓	Jessa et al. (1996), Plaznik et al. (1994), Przegalinsky et al. (1996)
Clycine site				
ACEA 1021	X-maze	=		Wiley et al. (1995)
HA 966	X-maze, SI, CT, USV			Trullas et al. (1989), Dunn et al. (1992), Anthony and Nevins (1993)
	ASP			Karcz et al. 1997
5,7 DCKA	Open field, CT, USV	↓	↓, =	Plaznik et al. (1994), Kehne et al. (1995), Corbett (1993)
7 CKA	ASP, CT	↓, =		Koek and Colpaert (1991), Anthony and Nevins (1993)
Cycloserine	ASP, X-maze	↓	=	Anthony and Nevins (1993), Karcz et al. (1997)
MDL	USV	↓	=, ↓	Kehne et al. (1995), Baron et al. (1997)
L-701,324	CT, X-maze	↓, =	=	Kotlinska and Liljequist (1998), Karcz et al. (1997)
ACPC	CT, X-maze, ASP	↓	=	Anthony and Nevins (1993), Przegalinsky et al. (1996), Karcz et al. (1997)
Polyamine site				
Ifenprodil	X-maze	↓	↑	Fraser et al. (1996)

Substances: MK 801, dizolcipine; PCP, phencyclidine; CPP, 3-(2-carboxy piperazine-4yl)-propyl-1-phosphonic-acid; CGS 19975, *cis*-4-phosphonomethyl-2-piperidine-carboxylkynurenate; AP5, 2 amino-5-phosphonoheptanoate; AP7, 2 amino-7-phosphonoheptanoate; HA 966, 3-amino-1-hydroxy-2-pyrrolidinone; 5,7 DCKA, 5,7-dichlorokynurenic acid; 7 CKA, 7-chlorokynurenic acid; MDL' 102,288, 5,7-dichloro-1,4-dihydro-(((4-((methoxycarbonyl)amino)-6-chloro-1H-indole-2-carboxylic acid; MDL" 100,458, (3(benzoylmethylamino)-6-chloro1H-indole-2-carboxylic acid; MDL"' 105,519, (*E*)-3-(2-phenyl-2-carboxyethenyl)-4,6-dichloro-1H-indole-2-carboxylic acid; L-701,324, 7-chloro-4-hydroxy-3-(3-phenoxy) phenyl-2(1H)-quinolone; ACPC, 1-aminocyclopropanecarboxylic acid; Memantine, amantine; LY326325; LY215490, 3*SR*, 4*aRS*, 6*RS*, 8*aRS*)-6-(2(1H-tetrazol-5-yl)ethyl)decahydro-isoquinoline-3-carboxylic acid; LY354740, 1*S*, 2*S*, 5*R*, 6*S*-2-aminobicyclo(3.1.0)hexane-2-6-dicarboxylate monohydrate; NBQX, dihydroxy-6-nitro-7-sulfamoyl-benzo(*F*)quinoxaline; MPEP, 2-methyl-6-(phenylethynyl)pyridine.
Anxiety tests: CT, conflict test; X-maze, elevated plus maze; USV, ultrasonic vocalisation; SI, social interaction test; ASP, acoustic startle paradigm.

These preclinical studies indicate that compounds active at NMDA, AMPA/kaïnate and metabotropic receptors might have anxiolytic properties. However, animal models for anxiety are only predictive to a limited extent and therefore can only be used as a rough screening method for the development of compounds with anxiolytic properties that are clinically effective. Common to conditioned as well as unconditioned tests is their reliance on motor behaviour (Dawson and Tricklebank, 1995). Some 'sedative-like' properties of the glutaminergic ligand could also result in the effects as presented in the tables. Drug induced non-specific enhancement or impairment of performance may confound anxiolytic drug effects. Specific tests on motor performance should be performed to distinguish these

effects from potential anxiogenic or anxiolytic effects. Not all authors have performed such independent tests for locomotor activity.

Furthermore, both conditioned as well as unconditioned animal models only measure direct potential anxiolytic effect. Delayed anxiolytic effects as observed with SSRIs were not found.

6. Clinical prospect of glutamate modulating compounds in anxiety disorder

Since the introduction of phencyclidine (PCP) in the late 1950s (Luby et al., 1959) antagonists of the NMDA glutamate receptor have been important tools for exploring the pathophysiology of neuro-psychiatric disorders. This interest has resulted in several clinical studies describing the effects of NMDA antagonists in normal, healthy subjects. Unfortunately, NMDA antagonists can profoundly affect behaviour and produce serious adverse effects (Willetts et al., 1994; Krystal et al., 1994, Danysz et al., 1995).

Studies in healthy human subjects suggest that NMDA antagonists produce disturbances in identity and perception. Cognitive disturbance and behavioural effects resembling schizophrenia have also been described (Javitt and Zukin, 1991). These behavioural effects could be explained by the dense cortical localisation of NMDA receptors (Krystal et al., 1999). These risks of NMDA-antagonists hampered clinical research. To prevent widespread excitotoxicity, NMDA channel blockers have been used in some clinical trials, e.g., in the treatment of Parkinson's disease or stroke (Brenner et al., 1989; Grotta et al., 1995). Some authors in the field of neuro-degenerative disease suggest that in the future it will be possible to develop NMDA receptor antagonists that are well tolerated (Parsons et al., 1999a,b). For the treatment of anxiety, excessive glutamate exposure in specific areas should be blocked, whereas normal glutaminergic neurotransmission should remain unaffected. Therefore, direct inhibition or excitation of the glutamate system is not a promising approach. By analogy, the GABA-benzodiazepine receptor complex has also several sites at which drugs can produce GABA-mediated effects (Johnston, 1996). Direct stimulation of the GABA-ion channel receptor by barbiturates has resulted in many central side effects. In contrast, benzodiazepines with their indirect mode of action modulate the endogenous GABA release (Haefely, 1990). This leads to a safer therapeutic approach. Analogous to the GABA system a more delicate regulation of the glutamate system would be desirable.

It is encouraging that different ways to fine-tune the glutaminergic system are emerging (Danysz et al., 1995; Parsons et al., 1999a,b). In addition to the NMDA-channel, the glycine site could also be a locus of attention (Dannhardt and Kohl, 1998; Kehne et al., 1995). Compounds acting at the AMPA receptor could also be promising (Karcz et al., 1995; Kotlinska and Liljequist, 1998a,b). An area that seems particularly fruitful for clinical application is the mGluRs system (Schoepp et al., 1999; Pin et al., 1999). mGluR agonists and antagonists have a wide variety of actions on central neurones, which are mediated by both voltage-gated and ligand-gated ion-channels. Activation of the different second messenger systems may have excitatory as well as inhibitory effects. Currently clinical studies evaluating anxiolytic properties of metabotropic agonists are under investigation.

Table 2
AMPA receptor antagonists

Substance	Anxiety test	Anxiety	Motor	Authors
Non-competitive antagonists				
LY326325	CT, X-maze	↓, ↑	=	Karcz et al. (1995), Kotlinska and Liljequist (1998)
LY215490	CT	↓		Benvenga et al. (1993)
NBQX	X-maze	↓, =		Karcz et al. (1995)

Table 3
Metabotropic receptor agonists and antagonist

Substance	Anxiety test	Anxiety	Motor	Authors
Agonists (mGlu 2)				
LY354740	ASP, X-maze	↓	=	Helton et al. (1997)
	CT	↓	↓	Klodzinska et al. (1999)
	SI	↓		Shekhar and Keim (2000)
Antagonists (mGlu 5)				
MPEP	X-maze, CT, SI	↓	=	Spooren et al. (2000)
	X-maze, CT	↓	=	Tatarczynska et al. (2001)
	CT	↓		Pilc et al. (2002)
	USV, ASP	↓	=	Brodkin et al. (2002)

7. Conclusion

In summary, although glutamate is a simple molecule, its action in the limbic system and areas concerning anxiety are complex and widespread (Danysz et al., 1995). These actions are mediated through different combinations of ionotropic and metabotropic glutamate receptors and potentially different sub-unit combinations. Preclinical studies indicated that compounds active at NMDA, AMPA/kaïnate and metabotropic receptors might have anxiolytic properties (see Tables 1–3). The major research effort so far has been directed towards the development of compounds which modulate the function of NMDA receptors by acting within the NMDA receptor complex. Anxiolytic properties and adverse effects due to muscle relaxant properties differ between the compounds used and the status of the activated sub-unit of the NMDA receptor. However, in general, the utility of NMDA and AMPA/kaïnate antagonists appeared to be greatly hampered by adverse effects because of interference with receptors throughout the whole CNS and body (Willetts et al., 1994; Krystal et al., 1994, Danysz et al., 1995). This has led to the conclusion that NMDA

receptor antagonism is not a valid therapeutic approach for the treatment of anxiety disorders. From the standpoint of novel therapeutic approaches to the treatment of anxiety a delicate regulation of the glutaminergic system is required. Normal glutaminergic neurotransmission, which takes place in virtually every synapse in the CNS, should be unaffected whereas the effects of excessive glutamate in specific areas should be blocked (Davidson, 2002; Maren, 1996; Parsons et al., 1999a,b).

It is encouraging that different ways to fine-tune the glutaminergic system are emerging. Modulators of the glycine site could be clinically useful, as well as compounds acting at the AMPA receptor (Dannhardt and Kohl, 1998a,b; Karcz et al., 1995; Kehne et al., 1995; Kotlinska and Liljequist, 1998a,b). Particularly promising are metabotropic glutamate agonists and antagonists. They represent a new and novel class of compounds with potential therapeutic efficacy in anxiety without some of the side effects associated with NMDA antagonists. The mGluR pharmacology is expanding rapidly. It is now apparent that several pre- and postsynaptic mechanisms exist by which in situ expressed mGluRs could modulate cell function in the CNS (Schoepp et al., 1999; Pin et al., 1999). To further explore mGluR function, the discovery of potent subtypes of selective mGluR compounds is needed. According to the authors, in future most therapeutic opportunities in anxiety disorders might arise from selective modulation of these metabotropic glutamate receptors. Furthermore it is beyond doubt that the actions of glutamate should not be considered in isolation at individual receptors, as glutamate acts at multiple receptors and is subjected to modulation from several sources. It is to be expected that modulators of glutamate activity may ultimately be of great clinical significance in the treatment of anxiety disorders and in psychiatry in general.

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Review

NMDA receptors as targets for drug action in neuropathic pain

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Abstract

Hyperalgesia and allodynia following peripheral tissue or nerve injury are not only due to an increase in the sensitivity of primary afferent nociceptors at the site of injury but also depend on NMDA receptor-mediated central changes in synaptic excitability. Functional inhibition of NMDA receptors can be achieved through actions at different recognition sites such as the primary transmitter site (competitive), strychnine-insensitive glycine site ($glycine_B$), polyamine site (NR2B selective) and phencyclidine site located inside the cationic channel. Unfortunately, most agents which completely block NMDA receptors cause numerous side effects such as memory impairment, psychotomimetic effects, ataxia and motor incoordination. There is now, however, considerable evidence that moderate affinity channel blockers, $glycine_B$ and NR2B selective antagonists show a much better profile in animal models than high affinity channel blockers and competitive NMDA receptor antagonists. These "therapeutically" safe NMDA receptor antagonists are also able to slow or prevent the development of opioid tolerance, indicating the utility of their combination with opioids in the treatment of chronic pain. The antinociceptive effects of NMDA receptor antagonists and opioids could be predicted to be synergistic and the presence of an NMDA receptor antagonist should block both the development of chronic pain states and inhibit the development of tolerance to the analgesic effects of morphine. Peripheral NMDA receptors offer a very attractive target for NMDA receptor antagonists that do not cross the blood brain barrier in inflammatory and visceral pain. Such agents might be predicted to be devoid of CNS side effects at doses producing powerful antinociception at peripheral NMDA receptors.

Keywords: NMDA receptor; Pain, chronic; Opioid tolerance

1. Glutamate in chronic pain

Despite intensive research on the neurobiological mechanisms of chronic pain, this therapeutic area remains one of the least satisfactorily covered by current drugs. Malfunctioning of glutamatergic neurotransmission has been implicated in a wide variety of neurological diseases such as acute stroke and trauma, chronic neurodegenerative diseases, epilepsy, schizophrenia and depression (see Parsons et al., 1998). Of particular relevance for this review is the involvement of glutamate in diseases reflecting long-term plastic changes in the central nervous system (CNS) such as chronic pain, opioid tolerance, dependence and addiction (Bennett, 2000).

Glutamate activates two major classes of receptors: ionotropic and metabotropic. Ionotropic receptors are classified into three major subclasses: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate and *N*-methyl-D-aspartate (NMDA). There is considerable preclinical evidence that hyperalgesia and allodynia following peripheral tissue or nerve injury is not only due to an increase in the sensitivity of primary afferent nociceptors at the site of injury but also depends on NMDA receptor-mediated central changes in synaptic excitability (Dickenson, 1990; Dickenson et al., 1997; Ren, 1994; Sandkuhler and Liu, 1998).

2. NMDA receptor antagonists

Functional inhibition of NMDA receptors can be achieved through actions at different recognition sites such as the primary transmitter site (competitive), strychnine-insensitive glycine site ($glycine_B$), polyamine site (NR2B selective) and phencyclidine site located inside the cationic channel (see Parsons et al., 1998). NMDA channel blockers act in an uncompetitive "use-dependent" manner, meaning that they only block the channel in the open state.

Unfortunately, antagonists which completely block NMDA receptors cause numerous side effects such as memory impairment, psychotomimetic effects, ataxia and

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motor incoordination, as they also impair normal synaptic transmission—a two-edged sword. The challenge has therefore been to develop NMDA receptor antagonists that prevent the *pathological* activation of NMDA receptors but allow their *physiological* activation.

2.1. Uncompetitive antagonists

It has been suggested that uncompetitive NMDA receptor antagonists with rapid unblocking kinetics but somewhat less pronounced voltage-dependency than Mg^{2+} should be able to antagonise the pathological effects of the sustained, but relatively small increases in extracellular glutamate concentration, but, like Mg^{2+}, leave the channel as a result of strong depolarisation following physiological activation (e.g. Parsons et al., 1999). Thus, uncompetitive NMDA receptor antagonists with moderate, rather than high affinity may be desirable. Memantine, amantadine, ketamine and dextromethorphan are clinically used agents which belong to this category (Hewitt, 2000; Schmid et al., 1999; Gordon et al., 1999). Others such as remacemide, (*S*)-alpha-phenyl-2-pyridineethanamine dihydrochloride (ARL 15896AR), bis-3flour-phenyl-propanamine (NPS-1506) and possibly the cannabinoid Dexanibinol (HU-211) are at different stages of clinical development (see Fig. 1 and Parsons et al., 1998).

Although the hypothesis underlying the ability of low affinity open channel blockers to differentiate between phasic physiological and tonic pathological activation of NMDA receptors during ischaemia has gained relatively wide acceptance (Chen et al., 1992; Rogawski, 1993; Kornhuber and Weller, 1997; Mealing et al., 1997; Parsons et al., 1999), it is still unclear how such compounds could differentiate between normal and abnormal synaptic activation of NMDA receptors. One possible explanation is that the forms of synaptic activity in the two states are different. Long-term potentiation is a biophysical model for the patterns of NMDA receptor-dependent synaptic activity underlying memory formation (Collingridge and Bliss, 1995; Herron et al., 1986) and is normally induced by delivering a high frequency tetanic stimulus (typically 100 Hz for 1 s). Under such conditions, drugs such as memantine can leave the open NMDA receptor channel during the stimulus burst. Indeed very high concentrations of memantine are required to block the induction of long-term potentiation both in vivo and in vitro (Barnes et al., 1996; Frankiewicz et al., 1996). Wind-up is a biophysical model for the patterns of synaptic activity underlying the induction of chronic pain states (Dickenson, 1990; Dickenson et al., 1997; Ren, 1994) and is normally induced by repetitive stimulation at much lower frequencies but for longer periods (typically 0.5 Hz for 5–10 s). Most NMDA receptor antagonists block this form of synaptic activity, including therapeutically relevant doses of moderate affinity uncompetitive antagonists. It should be noted, however, that the relevance of wind-up for the induction of chronic pain states has recently been questioned because wind-up, central sensitisation and hyperalgesia are not the same phenomena, even though they share some common properties (Herrero et al., 2000). Long-term depression is also an NMDA receptor-dependent biophysical model of synaptic plasticity that is induced by lower frequency stimulation over longer periods of time (Bear and Malenka, 1994; Malenka, 1994; Baudry, 1996) and requires less pro-

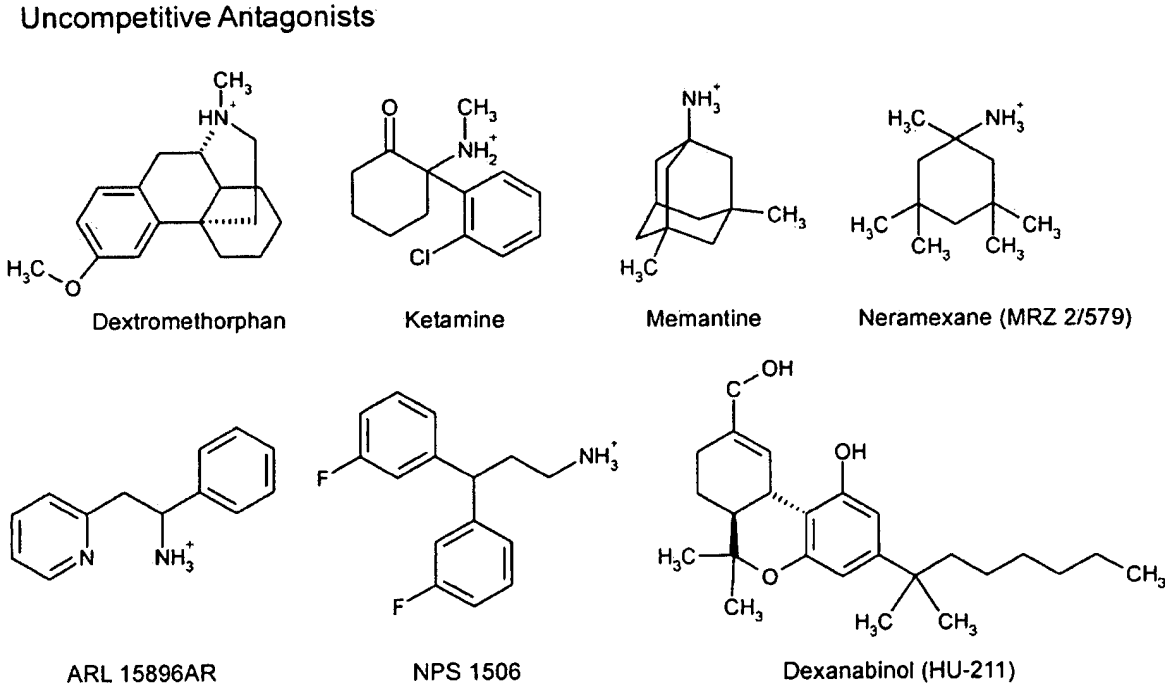

Fig. 1. Uncompetitive antagonists.

nounced increases in intracellular Ca^{2+} than long-term potentiation (Artola and Singer, 1993; Hansel et al., 1996). It seems likely that during the induction of wind-up or long-term depression for each successive NMDA-receptor mediated EPSP, the duration is too short and the degree of depolarisation too low to allow sufficient relief of blockade by low affinity open channel blockers. In other words, such compounds can block moderate NMDA receptor activation underlying, e.g. chronic pain states (modelled by wind-up) or the development of drug tolerance (possibly modelled by long-term depression) but do not block stronger NMDA receptor activation underlying memory formation (modelled by long-term potentiation).

Whatever the biophysical mechanism underlying this difference, therapeutically relevant doses of memantine selectively block formalin-induced tonic nociceptive responses in rats (Eisenberg et al., 1993) and produce a prophylactic antinociceptive effect against carrageenan-induced hyperalgesia (Neugebauer et al., 1993; Eisenberg et al., 1994) at doses devoid of side effects. Memantine also blocks and reverses thermal hyperalgesia and mechanical allodynia in rat models of painful mononeuropathy without obvious effects on motor reflexes following systemic (Carlton and Hargett, 1995; Eisenberg et al., 1995) and local spinal administration (Chaplan et al., 1997). Interestingly, in a recent study, memantine produced powerful inhibition of wind-up after selective ligation of the L5/L6 spinal nerves with little effect in sham controls in contrast to dizocilpine ((+) 5-methyl-10,11-dihydro-5*H*-dibenzo[a,d] cyclohepten 5,10 imine, (+)MK 801), which non-selectively blocked responses (Suzuki et al., 2001). Clinical trials with memantine in post herpetic neuralgia and diabetic neuropathic pain syndrome are presently underway. Ketamine is also effective in various animal models of hyperalgesia and allodynia and, like memantine, has been reported to have antinociceptive effects in some of these models at doses devoid of obvious side effects (Mao et al., 1993; Qian et al., 1996; Ren et al., 1992). Others, however, have reported that the effects of ketamine are only seen at doses producing ataxia (Chaplan et al., 1997; Laird et al., 1995; Yaksh, 1989). This may be related to its somewhat higher potency and associated slower unblocking kinetics.

2.2. Glycine site antagonists

Another promising target for NMDA receptor antagonism is the glycine$_B$ modulatory site (for review see Danysz and Parsons, 1998). Full antagonists of this site may offer a promising therapeutic profile due to their ability to increase NMDA receptor glycine-dependent desensitization. Prolonged repetitive activation of NMDA receptors would be effectively reduced at concentrations having little effect on more transient activation as the time course for full desensitization is quite long (tau several hundred milliseconds). This property may also allow such compounds to differentiate between various forms of NMDA receptor-mediated synaptic plasticity, e.g. block long-term depression and wind-up at concentrations having less effect on long-term potentiation. This idea is supported by recent data indicating that systemically active glycine$_B$ antagonists have good therapeutic indices following systemic administration in models of hyperalgesia (Vaccarino et al., 1993; Millan and Seguin, 1994; Laird et al., 1996; Quartaroli et al., 1999) as anxiolytics, possible anti-psychotomimetics, neuroprotective agents in models of focal ischaemia and trauma, and anti-epileptics (see Danysz and Parsons, 1998). In contrast to high affinity uncompetitive antagonists, glycine$_B$ antagonists do not have psychotomimetic effects (Löscher et al., 1994; Karcz-Kubicha et al., 1999) and have even been reported to possess antipsychotic activity (Bristow et al., 1995, 1996). They have no negative effects on learning at doses effectively blocking NMDA receptor in vivo (see Danysz and Parsons, 1998). Finally, unlike high affinity uncompetitive antagonists such as (+)-MK-801 and phencyclidine

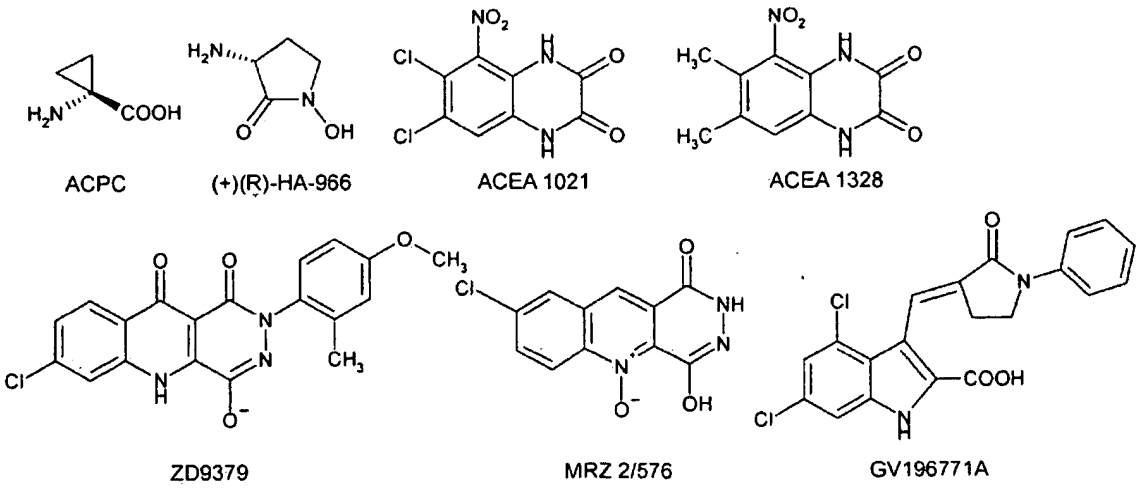

Fig. 2. Glycine site antagonists.

(PCP), even very high doses do not cause any neurodegenerative changes in the cingulate/retrosplenial cortex of rats (Chen et al., 1993; Hargreaves et al., 1993; Berger et al., 1994).

The glaxo compound GV 196771A is presently in phase I clinical trials for pain (see Fig. 2). This glycine$_B$ antagonists was effective in blocking the development of hyperalgesia following chronic sciatic nerve ligation in rats when given orally at 3 mg/kg twice daily for 10 days. GV 196771A also dose-dependently reversed established hyperalgesia for up to 8 h. The therapeutic profile was promising in as much as the second phase of formalin-induced hyperalgesia was antagonized with an ID_{50} of 0.6 mg/kg p.o. whereas 10 mg/kg had no effect on the first phase (Quartaroli et al., 1999). Other systemically active glycine$_B$ antagonists presently under development and with therapeutic potential in the treatment of pain include 5-nitro-6,7-dichloro-1,4-dihydro-2,3-quinoxalinedione (ACEA-1021, Licostinel, Lutfy et al., 1995, 1996), 7-Chloro-4-hydroxy-2-(4′-methoxyphenyl)-6′-methyl-1, 2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (ZD9379, Tatlisumak et al., 1998) and 8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazinol[4,5-]quinoline-5-oxide choline (MRZ 2/576, Parsons et al., 1997).

2.3. NR2B selective antagonists

Subtype selective agents may also offer a promising approach to minimize side effects as agents would not produce maximal inhibition of responses of neurones expressing heterogeneous receptors. Thus, cortical and hippocampal neurones express both NR2A and NR2B receptors in approximately similar proportions, but very little NR2C or NR2D. NR2B selective agents therefore block NMDA receptor mediated responses of such neurones to a maximal level of around 30–50% of control. The NR2B selective agent (1*S*,2*S*)-1-(4-hydroxy-phenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol (CP-101,606, see Fig. 3) has indeed been reported to be effective in suppressing hyperalgesia in animal models of chronic pain (carrageenan, 4-Phorbol-12-myristate-13-acetate (PMA), capsaicin and allodynia in neuropathic rats) at doses devoid of negative side effects in motor co-ordination or behaviour (Taniguchi et al., 1997; Boyce et al., 1999). A good separation was also reported for (*R*-(*R**,*S**)-(4-hydroxyphenyl)-methyl-4-(phenylmethyl)-1-piperidinepropanol ((±)-Ro 25-6981, Boyce et al., 1999) indicating that NR2B selective antagonists may also have clinical utility for the treatment of neuropathic and other pain conditions in man with a reduced side-effect profile. Unfortunately, several NR2B selective agents seem to block human ether-a-go-go-related gene (HERG)-mediated K(+) currents and may thereby produce severe side effects by increasing the cardiac Q/T interval.

3. Wind-up

More recent studies have addressed the issue whether it is possible to reproduce clinically the well characterized inhibition of wind-up seen with NMDA receptor antagonists in animal models. Dextromethorphan and ketamine, but not morphine significantly reduced both the area of secondary hyperalgesia and temporally summated 'wind-up-like pain' to repeated von Frey filament stimulation in healthy human volunteers with experimental first degree burn injury (Ilkjaer et al., 1997; Mikkelsen et al., 1999). Ketamine was also effective in reducing secondary hyperalgesia and allodynia induced by capsaicin in a double-blind, placebo-controlled, human experimental study (Andersen et al., 1996). Similar effects on "wind-up-like" pain were previously reported with i.v. ketamine and oral dextromethorphan in healthy volunteers, rating the intensity of summated second pain in response to repeated painful electric shocks and heat pulses (Price et al., 1994; Arendtnielsen et al., 1995).

4. Opioid tolerance

It is believed that phenomena such as sensitisation, tolerance and drug-dependence might also involve synaptic plasticity. In fact, numerous studies indicate that NMDA receptor antagonists block sensitisation to amphetamine and cocaine as well as tolerance and dependence to ethanol and opioids in animal models (Trujillo, 2000; Trujillo and Akil, 1991, 1995; Pasternak and Inturrisi, 1995; Mao, 1999). Recent studies indicate that the uncompetitive NMDA receptor antagonists dextromethorphan, memantine and MRZ 2/579 (1,3,3,5,5-pentamethylcyclohexylamine, neramexane) are not only able to prevent the development

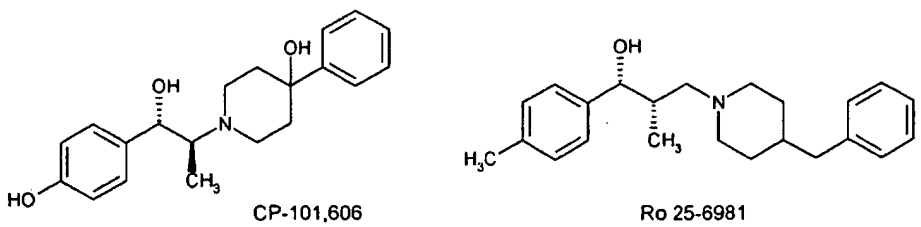

Fig. 3. NR2B selective anatagonists.

of morphine tolerance, but also reverse established tolerance even in the continuing presence of this opioid and prevent the expression of withdrawal symptoms in rats (Popik and Skolnick, 1996; Popik and Danysz, 1997; Popik and Kozela, 1999; Houghton et al., 2001). Moreover, acquisition of i.v. morphine self-administration in drug- and experimentally naive mice was inhibited by pretreatment with MRZ 2/579 (Semenova et al., 1999). Likewise, systemically active glycine$_B$ antagonists attenuate both physical dependence to morphine and the development of tolerance to the antinociceptive effects of opioids following repeated administration. Thus, 5-nitro-6,7-dimethyl-1,4-dihydro-2,3-quinoxalinedione (ACEA-1328) and ACEA-1021 completely blocked tolerance to morphine-induced antinociception in the tail flick test in mice, without affecting the basal nociceptive response or potentiating morphine-induced antinociceptive effects (Lutfy et al., 1995, 1996; Pasternak and Inturrisi, 1995). In contrast, the ability of MRZ 2/576 to attenuate morphine tolerance was associated with an increased morphine peak analgesia and duration in the tail-flick test (Popik et al., 1998; Belozertseva et al., 2000a,b). Administration of either (+)-(3*R*)-3-Amino-1-hydroxypyrrolidin-2-one ((+)-HA-966) or morphine alone was devoid of effects on the mechanical hyper responsiveness to von Frey hair stimulation in a model of chronic constriction injury whereas combined administration of (+)-HA-966 (2.5 mg/kg s.c.) and morphine (0.25, 0.5 and 1 mg/kg i.v.) dose-dependently increased the mechanical response thresholds (Christensen, 1999). This effect was naloxone-sensitive and was not accompanied by motor deficits (ibid.). When given alone 1-aminocyclopropane-carboxylic acid (ACPC, 50 and 150 mg/kg) had no analgesic actions in the tailflick assay and did not change morphine's potency in naive mice (Kolesnikov et al., 1994). However, chronic administration of this "functional" NMDA receptor antagonist both prevented and reversed morphine tolerance (ibid.). Also supportive for the role of NMDA receptors in opioid dependence is the finding that the opioid methadone (used in the therapy of addiction) also blocks NMDA receptors (Ebert et al., 1995) and that both the D- and L-isomer inhibit [^{3}H](+)-MK-801 binding to spinal cord membranes at therapeutically relevant concentrations: K_ds of 2.6 and 2.8 μM (Gorman et al., 1997). However, a recent paper concluded that NMDA antagonism does not contribute to the mechanism of D-methadone antinociception in vivo as both the antinociceptive and antagonism of responses to micro-iontophoretic NMDA were reversed by the opioid receptor antagonist naloxone (Chizh et al., 2000).

On a similar line, there are some indications that NMDA receptor activation may have a critical role in the mechanisms underlying the reduced effectiveness of opioids in chronic neuropathic pain states. For example, the antinociceptive effects of morphine are reduced in nerve-injured rats in the absence of daily exposure to morphine and this effect can be prevented by treatment with (+)-MK-801 (Mao et al., 1995). This may be due to the fact that NMDA receptor activation attenuates opioid receptor G protein coupling via activation of protein kinase C (Cai et al., 1997; Fan et al., 1998) and that opioid receptor activation increases NMDA receptor function via phosphorylation (Swope et al., 1999).

Taken together with the above mentioned effects of some NMDA receptor antagonists in models of chronic pain, these data indicate the utility of the combined use of therapeutically safe NMDA receptor antagonists with opioids in the treatment of chronic pain (Eide et al., 1995; Elliott et al., 1995; Bennett, 2000). The antinociceptive effects of NMDA receptor antagonists and opioids could be predicted to be synergistic and the presence of an NMDA receptor antagonist should block both the development of chronic pain states and inhibit the development of tolerance to the analgesic effects of morphine. Indeed, this approach has recently been pursued by Algos with morphidex (a combination of morphine and dextromethorphan), which is in phase III clinical trials for the treatment of chronic pain (see Parsons et al., 1998).

5. Peripheral NMDA receptors

Recent data indicate that peripheral NMDA receptors are also involved in inflammatory somatic and visceral pain. Peripheral glutamate receptors are associated with unmyelinated axons (Carlton et al., 1995) and the number of somatic sensory axons containing ionotropic glutamate receptors increases during peripheral sensitization due to inflammation (Carlton and Coggeshall, 1999; Coggeshall and Carlton, 1999). Subcutaneous administration of formalin into the plantar surface of rat hindpaws causes an increase in glutamate and aspartate concentrations in this tissue (Omote et al., 1998). Local injection of glutamatergic agonists to glabrous skin evokes pain-related behaviours in rats (Carlton et al., 1995; Jackson et al., 1995; Zhou et al., 1996) and peripherally administered glutamate receptor antagonists can prevent this effect as well as formalin or inflammation-induced hyperalgesia (Jackson et al., 1995; Davidson et al., 1997; Carlton et al., 1998; Davidson and Carlton, 1998). Similar effects have also been seen in experiments using local administration to the knee joint cavity in rats (Lawand et al., 1997). Local injection of ketamine into the skin on one calf of 10 healthy volunteers reduced primary and secondary hyperalgesia following a local experimental first degree burn injury, supporting the role of peripheral NMDA receptors in mediating secondary hyperalgesia in humans (Warncke et al., 1997)

Immunohistochemical studies indicate that NR1 subunits are expressed on the cell bodies and peripheral terminals of primary afferent nerves innervating the colon. Moreover, colorectal distension activation of single afferents in decentralised pelvic nerves was inhibited by me-

mantine, indicating that peripheral NMDA receptors are important in normal visceral pain transmission, and may provide a novel mechanism for development of peripheral sensitization and visceral hyperalgesia (McRoberts et al., 2001). Increases in blood pressure evoked by graded distension of the normal ureter was potently blocked by the glycine$_B$ antagonist MRZ 2/576, suggesting that NMDA receptors are involved in the processing of acute nociceptive inputs from viscera (Olivar and Laird, 1999). Moreover, the effective dose (ID_{50} = 0.2 mg/kg) was at least 10-fold lower than that known to antagonize NMDA receptors in the CNS (Parsons et al., 1997), indicating that peripheral NMDA receptors may also be involved in this effect.

6. Conclusions

(1) NMDA receptor antagonists which completely block NMDA receptors cause numerous side effects such as memory impairment, psychotomimetic effects, ataxia and motor incoordination, and they also impair normal synaptic transmission—a two-edged sword.

(2) The challenge has therefore been to develop NMDA receptor antagonists that prevent the pathological activation of NMDA receptors but allow their physiological activation.

(3) There is now considerable evidence that moderate affinity channel blockers, glycine$_B$ and NR2B selective antagonists show a much better profile in animal models of chronic pain than high affinity channel blockers and competitive NMDA receptor antagonists.

(4) These therapeutically safe NMDA receptor antagonists are also able to slow or prevent the development of opioid tolerance, indicating the utility of their combination with opioids in the treatment of chronic pain. The antinociceptive effects of NMDA receptor antagonists and opioids could be predicted to be synergistic and the presence of an NMDA receptor antagonist should block both the development of chronic pain states and inhibit the development of tolerance to the analgesic effects of morphine.

(5) The involvement of peripheral NMDA receptors in inflammatory and visceral nociception offers a very attractive target for NMDA receptor antagonists that do not cross the blood brain barrier. Such agents might be predicted to be devoid of CNS side effects at doses producing powerful antinociception at peripheral NMDA receptors.

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Review

NMDA receptors as targets for drug action in neuropathic pain

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Abstract

Hyperalgesia and allodynia following peripheral tissue or nerve injury are not only due to an increase in the sensitivity of primary afferent nociceptors at the site of injury but also depend on NMDA receptor-mediated central changes in synaptic excitability. Functional inhibition of NMDA receptors can be achieved through actions at different recognition sites such as the primary transmitter site (competitive), strychnine-insensitive glycine site ($glycine_B$), polyamine site (NR2B selective) and phencyclidine site located inside the cationic channel. Unfortunately, most agents which completely block NMDA receptors cause numerous side effects such as memory impairment, psychotomimetic effects, ataxia and motor incoordination. There is now, however, considerable evidence that moderate affinity channel blockers, $glycine_B$ and NR2B selective antagonists show a much better profile in animal models than high affinity channel blockers and competitive NMDA receptor antagonists. These "therapeutically" safe NMDA receptor antagonists are also able to slow or prevent the development of opioid tolerance, indicating the utility of their combination with opioids in the treatment of chronic pain. The antinociceptive effects of NMDA receptor antagonists and opioids could be predicted to be synergistic and the presence of an NMDA receptor antagonist should block both the development of chronic pain states and inhibit the development of tolerance to the analgesic effects of morphine. Peripheral NMDA receptors offer a very attractive target for NMDA receptor antagonists that do not cross the blood brain barrier in inflammatory and visceral pain. Such agents might be predicted to be devoid of CNS side effects at doses producing powerful antinociception at peripheral NMDA receptors.

Keywords: NMDA receptor; Pain, chronic; Opioid tolerance

1. Glutamate in chronic pain

Despite intensive research on the neurobiological mechanisms of chronic pain, this therapeutic area remains one of the least satisfactorily covered by current drugs. Malfunctioning of glutamatergic neurotransmission has been implicated in a wide variety of neurological diseases such as acute stroke and trauma, chronic neurodegenerative diseases, epilepsy, schizophrenia and depression (see Parsons et al., 1998). Of particular relevance for this review is the involvement of glutamate in diseases reflecting long-term plastic changes in the central nervous system (CNS) such as chronic pain, opioid tolerance, dependence and addiction (Bennett, 2000).

Glutamate activates two major classes of receptors: ionotropic and metabotropic. Ionotropic receptors are classified into three major subclasses: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate and *N*-methyl-D-aspartate (NMDA). There is considerable preclinical evidence that hyperalgesia and allodynia following peripheral tissue or nerve injury is not only due to an increase in the sensitivity of primary afferent nociceptors at the site of injury but also depends on NMDA receptor-mediated central changes in synaptic excitability (Dickenson, 1990; Dickenson et al., 1997; Ren, 1994; Sandkuhler and Liu, 1998).

2. NMDA receptor antagonists

Functional inhibition of NMDA receptors can be achieved through actions at different recognition sites such as the primary transmitter site (competitive), strychnine-insensitive glycine site ($glycine_B$), polyamine site (NR2B selective) and phencyclidine site located inside the cationic channel (see Parsons et al., 1998). NMDA channel blockers act in an uncompetitive "use-dependent" manner, meaning that they only block the channel in the open state.

Unfortunately, antagonists which completely block NMDA receptors cause numerous side effects such as memory impairment, psychotomimetic effects, ataxia and

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motor incoordination, as they also impair normal synaptic transmission—a two-edged sword. The challenge has therefore been to develop NMDA receptor antagonists that prevent the *pathological* activation of NMDA receptors but allow their *physiological* activation.

2.1. Uncompetitive antagonists

It has been suggested that uncompetitive NMDA receptor antagonists with rapid unblocking kinetics but somewhat less pronounced voltage-dependency than Mg^{2+} should be able to antagonise the pathological effects of the sustained, but relatively small increases in extracellular glutamate concentration, but, like Mg^{2+}, leave the channel as a result of strong depolarisation following physiological activation (e.g. Parsons et al., 1999). Thus, uncompetitive NMDA receptor antagonists with moderate, rather than high affinity may be desirable. Memantine, amantadine, ketamine and dextromethorphan are clinically used agents which belong to this category (Hewitt, 2000; Schmid et al., 1999; Gordon et al., 1999). Others such as remacemide, (*S*)-alpha-phenyl-2-pyridineethanamine dihydrochloride (ARL 15896AR), bis-3flour-phenyl-propanamine (NPS-1506) and possibly the cannabinoid Dexanibinol (HU-211) are at different stages of clinical development (see Fig. 1 and Parsons et al., 1998).

Although the hypothesis underlying the ability of low affinity open channel blockers to differentiate between phasic physiological and tonic pathological activation of NMDA receptors during ischaemia has gained relatively wide acceptance (Chen et al., 1992; Rogawski, 1993; Kornhuber and Weller, 1997; Mealing et al., 1997; Parsons et al., 1999), it is still unclear how such compounds could differentiate between normal and abnormal synaptic activation of NMDA receptors. One possible explanation is that the forms of synaptic activity in the two states are different. Long-term potentiation is a biophysical model for the patterns of NMDA receptor-dependent synaptic activity underlying memory formation (Collingridge and Bliss, 1995; Herron et al., 1986) and is normally induced by delivering a high frequency tetanic stimulus (typically 100 Hz for 1 s). Under such conditions, drugs such as memantine can leave the open NMDA receptor channel during the stimulus burst. Indeed very high concentrations of memantine are required to block the induction of long-term potentiation both in vivo and in vitro (Barnes et al., 1996; Frankiewicz et al., 1996). Wind-up is a biophysical model for the patterns of synaptic activity underlying the induction of chronic pain states (Dickenson, 1990; Dickenson et al., 1997; Ren, 1994) and is normally induced by repetitive stimulation at much lower frequencies but for longer periods (typically 0.5 Hz for 5–10 s). Most NMDA receptor antagonists block this form of synaptic activity, including therapeutically relevant doses of moderate affinity uncompetitive antagonists. It should be noted, however, that the relevance of wind-up for the induction of chronic pain states has recently been questioned because wind-up, central sensitisation and hyperalgesia are not the same phenomena, even though they share some common properties (Herrero et al., 2000). Long-term depression is also an NMDA receptor-dependent biophysical model of synaptic plasticity that is induced by lower frequency stimulation over longer periods of time (Bear and Malenka, 1994; Malenka, 1994; Baudry, 1996) and requires less pro-

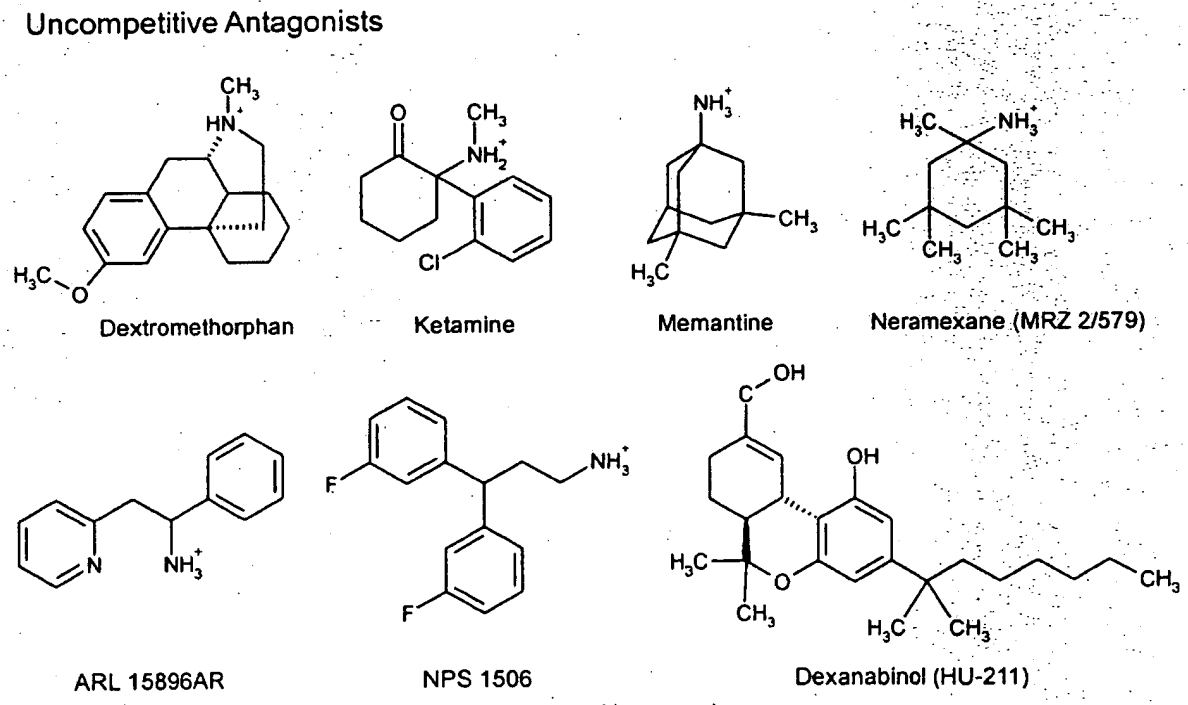

Fig. 1. Uncompetitive antagonists.

nounced increases in intracellular Ca^{2+} than long-term potentiation (Artola and Singer, 1993; Hansel et al., 1996). It seems likely that during the induction of wind-up or long-term depression for each successive NMDA-receptor mediated EPSP, the duration is too short and the degree of depolarisation too low to allow sufficient relief of blockade by low affinity open channel blockers. In other words, such compounds can block moderate NMDA receptor activation underlying, e.g. chronic pain states (modelled by wind-up) or the development of drug tolerance (possibly modelled by long-term depression) but do not block stronger NMDA receptor activation underlying memory formation (modelled by long-term potentiation).

Whatever the biophysical mechanism underlying this difference, therapeutically relevant doses of memantine selectively block formalin-induced tonic nociceptive responses in rats (Eisenberg et al., 1993) and produce a prophylactic antinociceptive effect against carrageenan-induced hyperalgesia (Neugebauer et al., 1993; Eisenberg et al., 1994) at doses devoid of side effects. Memantine also blocks and reverses thermal hyperalgesia and mechanical allodynia in rat models of painful mononeuropathy without obvious effects on motor reflexes following systemic (Carlton and Hargett, 1995; Eisenberg et al., 1995) and local spinal administration (Chaplan et al., 1997). Interestingly, in a recent study, memantine produced powerful inhibition of wind-up after selective ligation of the L5/L6 spinal nerves with little effect in sham controls in contrast to dizocilpine ((+) 5-methyl-10,11-dihydro-5*H*-dibenzo[a,d] cyclohepten 5,10 imine, (+)MK 801), which non-selectively blocked responses (Suzuki et al., 2001). Clinical trials with memantine in post herpetic neuralgia and diabetic neuropathic pain syndrome are presently underway. Ketamine is also effective in various animal models of hyperalgesia and allodynia and, like memantine, has been reported to have antinociceptive effects in some of these models at doses devoid of obvious side effects (Mao et al., 1993; Qian et al., 1996; Ren et al., 1992). Others, however, have reported that the effects of ketamine are only seen at doses producing ataxia (Chaplan et al., 1997; Laird et al., 1995; Yaksh, 1989). This may be related to its somewhat higher potency and associated slower unblocking kinetics.

2.2. Glycine site antagonists

Another promising target for NMDA receptor antagonism is the glycine$_B$ modulatory site (for review see Danysz and Parsons, 1998). Full antagonists of this site may offer a promising therapeutic profile due to their ability to increase NMDA receptor glycine-dependent desensitization. Prolonged repetitive activation of NMDA receptors would be effectively reduced at concentrations having little effect on more transient activation as the time course for full desensitization is quite long (tau several hundred milliseconds). This property may also allow such compounds to differentiate between various forms of NMDA receptor-mediated synaptic plasticity, e.g. block long-term depression and wind-up at concentrations having less effect on long-term potentiation. This idea is supported by recent data indicating that systemically active glycine$_B$ antagonists have good therapeutic indices following systemic administration in models of hyperalgesia (Vaccarino et al., 1993; Millan and Seguin, 1994; Laird et al., 1996; Quartaroli et al., 1999) as anxiolytics, possible anti-psychotomimetics, neuroprotective agents in models of focal ischaemia and trauma, and anti-epileptics (see Danysz and Parsons, 1998). In contrast to high affinity uncompetitive antagonists, glycine$_B$ antagonists do not have psychotomimetic effects (Löscher et al., 1994; Karcz-Kubicha et al., 1999) and have even been reported to possess antipsychotic activity (Bristow et al., 1995, 1996). They have no negative effects on learning at doses effectively blocking NMDA receptor in vivo (see Danysz and Parsons, 1998). Finally, unlike high affinity uncompetitive antagonists such as (+)-MK-801 and phencyclidine

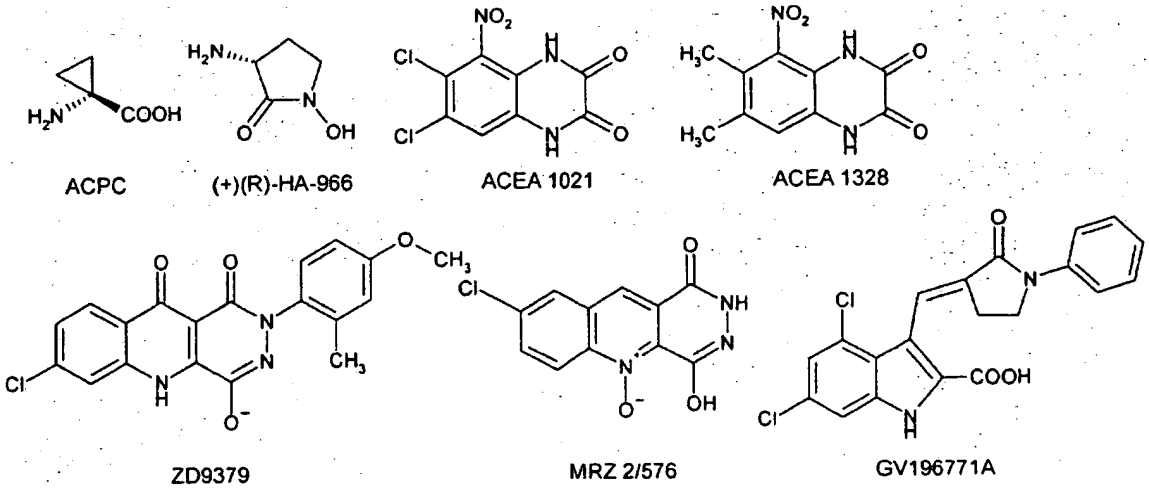

Fig. 2. Glycine site antagonists.

(PCP), even very high doses do not cause any neurodegenerative changes in the cingulate/retrosplenial cortex of rats (Chen et al., 1993; Hargreaves et al., 1993; Berger et al., 1994).

The glaxo compound GV 196771A is presently in phase I clinical trials for pain (see Fig. 2). This glycine$_B$ antagonists was effective in blocking the development of hyperalgesia following chronic sciatic nerve ligation in rats when given orally at 3 mg/kg twice daily for 10 days. GV 196771A also dose-dependently reversed established hyperalgesia for up to 8 h. The therapeutic profile was promising in as much as the second phase of formalin-induced hyperalgesia was antagonized with an ID_{50} of 0.6 mg/kg p.o. whereas 10 mg/kg had no effect on the first phase (Quartaroli et al., 1999). Other systemically active glycine$_B$ antagonists presently under development and with therapeutic potential in the treatment of pain include 5-nitro-6,7-dichloro-1,4-dihydro-2,3-quinoxalinedione (ACEA-1021, Licostinel, Lutfy et al., 1995, 1996), 7-Chloro-4-hydroxy-2-(4′-methoxyphenyl)-6′-methyl-1, 2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (ZD9379, Tatlisumak et al., 1998) and 8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazinol[4,5-]quinoline-5-oxide choline (MRZ 2/576, Parsons et al., 1997).

2.3. NR2B selective antagonists

Subtype selective agents may also offer a promising approach to minimize side effects as agents would not produce maximal inhibition of responses of neurones expressing heterogeneous receptors. Thus, cortical and hippocampal neurones express both NR2A and NR2B receptors in approximately similar proportions, but very little NR2C or NR2D. NR2B selective agents therefore block NMDA receptor mediated responses of such neurones to a maximal level of around 30–50% of control. The NR2B selective agent (1*S*,2*S*)-1-(4-hydroxy-phenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol (CP-101,606, see Fig. 3) has indeed been reported to be effective in suppressing hyperalgesia in animal models of chronic pain (carrageenan, 4-Phorbol-12-myristate-13-acetate (PMA), capsaicin and allodynia in neuropathic rats) at doses devoid of negative side effects in motor co-ordination or behaviour (Taniguchi et al., 1997; Boyce et al., 1999). A good separation was also reported for (*R*-(*R**,*S**)-(4-hydroxyphenyl)-methyl-4-(phenylmethyl)-1-piperidinepropanol ((±)-Ro 25-6981, Boyce et al., 1999) indicating that NR2B selective antagonists may also have clinical utility for the treatment of neuropathic and other pain conditions in man with a reduced side-effect profile. Unfortunately, several NR2B selective agents seem to block human ether-a-go-go-related gene (HERG)-mediated K(+) currents and may thereby produce severe side effects by increasing the cardiac Q/T interval.

3. Wind-up

More recent studies have addressed the issue whether it is possible to reproduce clinically the well characterized inhibition of wind-up seen with NMDA receptor antagonists in animal models. Dextromethorphan and ketamine, but not morphine significantly reduced both the area of secondary hyperalgesia and temporally summated 'wind-up-like pain' to repeated von Frey filament stimulation in healthy human volunteers with experimental first degree burn injury (Ilkjaer et al., 1997; Mikkelsen et al., 1999). Ketamine was also effective in reducing secondary hyperalgesia and allodynia induced by capsaicin in a double-blind, placebo-controlled, human experimental study (Andersen et al., 1996). Similar effects on "wind-up-like" pain were previously reported with i.v. ketamine and oral dextromethorphan in healthy volunteers, rating the intensity of summated second pain in response to repeated painful electric shocks and heat pulses (Price et al., 1994; Arendtnielsen et al., 1995).

4. Opioid tolerance

It is believed that phenomena such as sensitisation, tolerance and drug-dependence might also involve synaptic plasticity. In fact, numerous studies indicate that NMDA receptor antagonists block sensitisation to amphetamine and cocaine as well as tolerance and dependence to ethanol and opioids in animal models (Trujillo, 2000; Trujillo and Akil, 1991, 1995; Pasternak and Inturrisi, 1995; Mao, 1999). Recent studies indicate that the uncompetitive NMDA receptor antagonists dextromethorphan, memantine and MRZ 2/579 (1,3,3,5,5-pentamethylcyclohexylamine, neramexane) are not only able to prevent the development

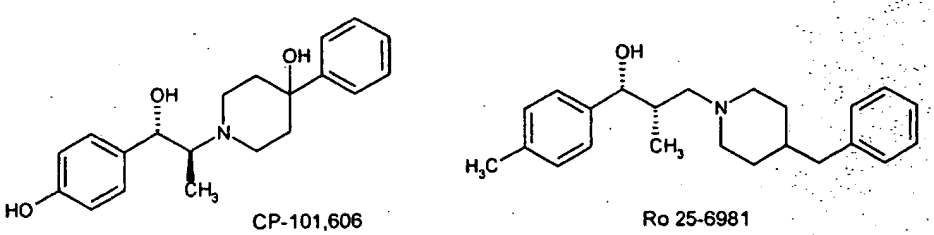

Fig. 3. NR2B selective anatagonists.

of morphine tolerance, but also reverse established tolerance even in the continuing presence of this opioid and prevent the expression of withdrawal symptoms in rats (Popik and Skolnick, 1996; Popik and Danysz, 1997; Popik and Kozela, 1999; Houghton et al., 2001). Moreover, acquisition of i.v. morphine self-administration in drug- and experimentally naive mice was inhibited by pretreatment with MRZ 2/579 (Semenova et al., 1999). Likewise, systemically active glycine$_B$ antagonists attenuate both physical dependence to morphine and the development of tolerance to the antinociceptive effects of opioids following repeated administration. Thus, 5-nitro-6,7-dimethyl-1,4-dihydro-2,3-quinoxalinedione (ACEA-1328) and ACEA-1021 completely blocked tolerance to morphine-induced antinociception in the tail flick test in mice, without affecting the basal nociceptive response or potentiating morphine-induced antinociceptive effects (Lutfy et al., 1995, 1996; Pasternak and Inturrisi, 1995). In contrast, the ability of MRZ 2/576 to attenuate morphine tolerance was associated with an increased morphine peak analgesia and duration in the tail-flick test (Popik et al., 1998; Belozertseva et al., 2000a,b). Administration of either (+)-(3*R*)-3-Amino-1-hydroxypyrrolidin-2-one ((+)-HA-966) or morphine alone was devoid of effects on the mechanical hyper responsiveness to von Frey hair stimulation in a model of chronic constriction injury whereas combined administration of (+)-HA-966 (2.5 mg/kg s.c.) and morphine (0.25, 0.5 and 1 mg/kg i.v.) dose-dependently increased the mechanical response thresholds (Christensen, 1999). This effect was naloxone-sensitive and was not accompanied by motor deficits (ibid.). When given alone 1-aminocyclopropane-carboxylic acid (ACPC, 50 and 150 mg/kg) had no analgesic actions in the tailflick assay and did not change morphine's potency in naive mice (Kolesnikov et al., 1994). However, chronic administration of this "functional" NMDA receptor antagonist both prevented and reversed morphine tolerance (ibid.). Also supportive for the role of NMDA receptors in opioid dependence is the finding that the opioid methadone (used in the therapy of addiction) also blocks NMDA receptors (Ebert et al., 1995) and that both the D- and L-isomer inhibit [^{3}H](+)-MK-801 binding to spinal cord membranes at therapeutically relevant concentrations: K_ds of 2.6 and 2.8 μM (Gorman et al., 1997). However, a recent paper concluded that NMDA antagonism does not contribute to the mechanism of D-methadone antinociception in vivo as both the antinociceptive and antagonism of responses to micro-iontophoretic NMDA were reversed by the opioid receptor antagonist naloxone (Chizh et al., 2000).

On a similar line, there are some indications that NMDA receptor activation may have a critical role in the mechanisms underlying the reduced effectiveness of opioids in chronic neuropathic pain states. For example, the antinociceptive effects of morphine are reduced in nerve-injured rats in the absence of daily exposure to morphine and this effect can be prevented by treatment with (+)-MK-801 (Mao et al., 1995). This may be due to the fact that NMDA receptor activation attenuates opioid receptor G protein coupling via activation of protein kinase C (Cai et al., 1997; Fan et al., 1998) and that opioid receptor activation increases NMDA receptor function via phosphorylation (Swope et al., 1999).

Taken together with the above mentioned effects of some NMDA receptor antagonists in models of chronic pain, these data indicate the utility of the combined use of therapeutically safe NMDA receptor antagonists with opioids in the treatment of chronic pain (Eide et al., 1995; Elliott et al., 1995; Bennett, 2000). The antinociceptive effects of NMDA receptor antagonists and opioids could be predicted to be synergistic and the presence of an NMDA receptor antagonist should block both the development of chronic pain states and inhibit the development of tolerance to the analgesic effects of morphine. Indeed, this approach has recently been pursued by Algos with morphidex (a combination of morphine and dextromethorphan), which is in phase III clinical trials for the treatment of chronic pain (see Parsons et al., 1998).

5. Peripheral NMDA receptors

Recent data indicate that peripheral NMDA receptors are also involved in inflammatory somatic and visceral pain. Peripheral glutamate receptors are associated with unmyelinated axons (Carlton et al., 1995) and the number of somatic sensory axons containing ionotropic glutamate receptors increases during peripheral sensitization due to inflammation (Carlton and Coggeshall, 1999; Coggeshall and Carlton, 1999). Subcutaneous administration of formalin into the plantar surface of rat hindpaws causes an increase in glutamate and aspartate concentrations in this tissue (Omote et al., 1998). Local injection of glutamatergic agonists to glabrous skin evokes pain-related behaviours in rats (Carlton et al., 1995; Jackson et al., 1995; Zhou et al., 1996) and peripherally administered glutamate receptor antagonists can prevent this effect as well as formalin or inflammation-induced hyperalgesia (Jackson et al., 1995; Davidson et al., 1997; Carlton et al., 1998; Davidson and Carlton, 1998). Similar effects have also been seen in experiments using local administration to the knee joint cavity in rats (Lawand et al., 1997). Local injection of ketamine into the skin on one calf of 10 healthy volunteers reduced primary and secondary hyperalgesia following a local experimental first degree burn injury, supporting the role of peripheral NMDA receptors in mediating secondary hyperalgesia in humans (Warncke et al., 1997)

Immunohistochemical studies indicate that NR1 subunits are expressed on the cell bodies and peripheral terminals of primary afferent nerves innervating the colon. Moreover, colorectal distension activation of single afferents in decentralised pelvic nerves was inhibited by me-

mantine, indicating that peripheral NMDA receptors are important in normal visceral pain transmission, and may provide a novel mechanism for development of peripheral sensitization and visceral hyperalgesia (McRoberts et al., 2001). Increases in blood pressure evoked by graded distension of the normal ureter was potently blocked by the glycine$_B$ antagonist MRZ 2/576, suggesting that NMDA receptors are involved in the processing of acute nociceptive inputs from viscera (Olivar and Laird, 1999). Moreover, the effective dose (ID_{50} = 0.2 mg/kg) was at least 10-fold lower than that known to antagonize NMDA receptors in the CNS (Parsons et al., 1997), indicating that peripheral NMDA receptors may also be involved in this effect.

6. Conclusions

(1) NMDA receptor antagonists which completely block NMDA receptors cause numerous side effects such as memory impairment, psychotomimetic effects, ataxia and motor incoordination, and they also impair normal synaptic transmission—a two-edged sword.

(2) The challenge has therefore been to develop NMDA receptor antagonists that prevent the pathological activation of NMDA receptors but allow their physiological activation.

(3) There is now considerable evidence that moderate affinity channel blockers, glycine$_B$ and NR2B selective antagonists show a much better profile in animal models of chronic pain than high affinity channel blockers and competitive NMDA receptor antagonists.

(4) These therapeutically safe NMDA receptor antagonists are also able to slow or prevent the development of opioid tolerance, indicating the utility of their combination with opioids in the treatment of chronic pain. The antinociceptive effects of NMDA receptor antagonists and opioids could be predicted to be synergistic and the presence of an NMDA receptor antagonist should block both the development of chronic pain states and inhibit the development of tolerance to the analgesic effects of morphine.

(5) The involvement of peripheral NMDA receptors in inflammatory and visceral nociception offers a very attractive target for NMDA receptor antagonists that do not cross the blood brain barrier. Such agents might be predicted to be devoid of CNS side effects at doses producing powerful antinociception at peripheral NMDA receptors.

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N-Methyl-D-Aspartate Receptor (NMDA) Antagonists as Potential Pain Therapeutics

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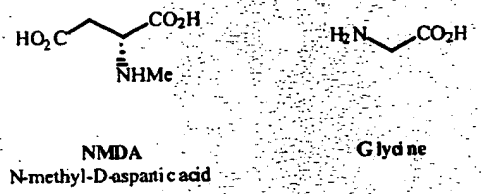
Abstract: NMDA receptors are known to be involved in nociceptive transmission and pain processing. Many structurally diverse NMDA antagonists have been reported to have activity in both animal models and clinical models of neuropathic pain. Untoward side effects such as ataxia and sedation have severely limited the clinical uses of this class of potential therapeutics. However, antagonists at the glycine-site, NR2B sites and weak-binding channel blockers have demonstrated an improved side effect profile in animal models of pain. These types of compounds may hold potential promise for future pain therapies. This review covers reported pain data surrounding representative examples of NMDA antagonists and provides a current assessment of potential clinical utility.

INTRODUCTION

Based on considerable evidence, it has been recognized that N-methyl-D-aspartate (NMDA) receptors are partially responsible for the onset and maintenance of neuropathic pain [1]. This link has been established from *in vitro* pharmacology studies, behavioral models, animal models of neuropathic pain and clinical trial data. However, side effects such as ataxia and sedation have severely limited the clinical use of NMDA antagonists [2]. A deeper understanding of NMDA receptor pharmacology (*e.g.* identification of multiple antagonist binding sites and subtype specificity) has led to the second-generation antagonists (glycine-site, NR2B, weak affinity channel blockers) that may hold greater promise for future pain therapies with a more tolerable side effect profile [3]. The purpose of this review is to summarize the relevant *in vitro* pharmacological data, *in vivo* pain data and clinical trial-pain data from representative examples of the four classes of NMDA antagonists, which are: a) non-competitive channel blockers, b) competitive glutamate-site antagonists, c) competitive glycine-site antagonists and d) non-competitive antagonists of allosteric nature acting at sites linked to polyamine or ifenprodil action.

Glutamate is a critical excitatory amino acid that is important for nociceptive processing in the spinal cord [4]. Glutamate receptors can be divided into two families, ionotropic (ligand-gated ion channels) and metabotropic (G-protein coupled) [5]. Ionotropic receptors are further sub-divided into three classes: α-amino-3-hydroxy-5-methyl-4-isoxazole-proprionic acid receptors (AMPA), kainate receptors (KA) and NMDA receptors [6,7]. Among the ionotropic glutamate receptors, NMDA receptors are unique in that channel activation requires not only interaction with glutamate, but also another amino acid, glycine [8,9,10]. The glycine binding-site is distinct from the glutamate binding site, and glycine thus is a true co-agonist. Furthermore, modulatory ligands, like polyamines can alter the response of NMDA receptors to both glutamate and glycine [11-14].



NMDA
N-methyl-D-aspartic acid

Glycine

Fig. (1). Excitatory amino acids acting at the NMDA receptor.

MOLECULAR STRUCTURE OF NMDA RECEPTORS

Functional NMDA receptors are heteromultimeric complexes likely to consist of several subunits [15,16]. Three distinct gene families have been identified that encode these subunits. The NR1 subunit is encoded by a single gene. However, because this gene can be spliced at three sites, the NR1 protein can exist as eight different isoforms [17]. The NR2 subunit is encoded by four different genes generating the NR2A, NR2B, NR2C and NR2D subunits, respectively [18, 19]. An NR3 subunit is also known and is proposed to play a regulatory role in development, perhaps in a protective sense [20]. The most common combination of these subunits in a functional NMDA receptor appears to be a complex of two NR1 subunits and two NR2 subunits [21].

Ligand sites of interaction are well-characterized with respect to receptor subtype. The glycine binding site is located on the NR1 subunit, whereas the glutamate site is located on the NR2 subunit [22-24]. It is also believed that polyamine-site antagonists such as ifenprodil bind at the NR2B subunit [25]. There is a reported high-affinity Zn^{2+} binding site located on the NR2A subunit that shares similarities to the ifenprodil site in the NR2B subunit [26]. All of the subtypes are heterogeneously distributed in brain and CNS [27, 28, 29]. The implications of subtype distribution are not yet fully understood. The past few years have witnessed huge advances in our understanding of the molecular structure of ionotropic glutamate receptors (iGluRs), including the NMDA receptor. These new insights have helped dramatically in the understanding of ligand interaction and it appears appropriate to briefly discuss them

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here. For further reading the reader is referred to a recent review on the topic [30].

The extracellular regions of all iGluR subunits comprise the extracellular N-terminus of approximately 500 residues and the region between the second and third transmembrane region. Inserted between these two extracellular regions is the pore-forming region consisting of two transmembrane regions separated by a hairpin-formed P-loop that forms the narrowest part of the actual ion channel (selectivity filter). A third transmembrane region and a large intracellular C-terminus follow the second extracellular region [31,32]. Interestingly, the two extracellular regions have similarity to periplasmic binding proteins (PBP) found in some bacteria [23, 33-35]. The most distal part of the N-terminus, comprising approximately the first 350 residues of iGluR subunits, has similarities to a PBP that in bacteria binds the amino–acids leucine, isoleucine, and valine [36]. In iGluRs this region was thus initially referred to as the LIVBP-like region. However, because the actual similarities between this region and the LIVBP of bacteria are weak, most authors prefer at present the more neutral label ATD (amino-terminal domain) for this region [37, 38]. The remainder of the N-terminus and the extracellular region between transmembrane 2 and 3 of all iGluR subunits has similarities to several PBP, with the strongest similarity to the bacterial glutamine binding protein [39]. In iGluR subunits this region is thus referred to as the GlnBP-like region. Note that earlier literature referred to this region as the LAOBP-like region, from the bacterial lysine, arginine, ornithine binding protein [40].

Agonists bind to iGluRs at the GlnBP-like region [30, 31]. At NMDA receptors, glutamate binds to the GlnBP-like region of the NR2 subunits, and the co-agonist glycine binds to the GlnBP-like region of NR1 [23, 24]. Opening of NMDA receptor ion channels is likely to require occupancy of all GlnBP-like regions of the receptor by the respective agonist, (*i.e.* binding of two molecules of glycine and glutamate each to one NMDA channel) [41]. Competitive antagonists interfere with binding of glycine to the GlnBP-like region of NR1 or with binding of glutamate to the GlnBP-like region of NR2.

Ligand binding to the ATD region does not activate iGluRs, but has modulatory, allosteric roles [37, 38]. The ATD of NR2A has been shown to influence NMDA receptor desensitization [42, 43], and this effect seems, at least partially, to be mediated by high-affinity binding of Zn^{2+} to the ATD of NR2A [26]. Likewise, binding of ifenprodil to the ATD of NR2B inhibits activity of NMDA receptors containing this subunit [25]. No ligands have yet been identified that bind to the ATD of NR2C or NR2D subunits, but such ligands may hold potential for subtype-specific modulation of NMDA receptors.

From crystallization studies it was known that in bacteria the binding proteins form a "clam-shell" like structure, that can exist in an "open" and "closed" configuration, with the ligands stabilizing the closed configuration [35, 36, 39, 40]. A similar scenario was thus envisioned for ligand action at iGluRs [23, 33]. Crystallization studies of the isolated GlnBP-like regions of iGluRs [44], including the GlnBP-like region of the NR1 subunit [45, 46], have largely confirmed this picture. Interestingly, crystals of the GlnBP-like region of NR1 grown in the presence of the agonist glycine or several partial agonists result in a "closed" clam-shell structure, whereas crystals grown in the presence of a glycine site antagonist reveal an "open" clam-shell structure [46]. These recent studies are likely to herald a new era of computational approaches to the development of NMDA receptor antagonists and modulators.

PHYSIOLOGICAL ROLE OF NMDA RECEPTORS

NMDA receptors play a key role in integrating and transforming fast synaptic signaling into slower dynamic modifications at the intracellular level. This integratory function is in large part achieved by two biophysical properties of open NMDA receptors: voltage-dependent block by extracellular magnesium ions, and permeability to

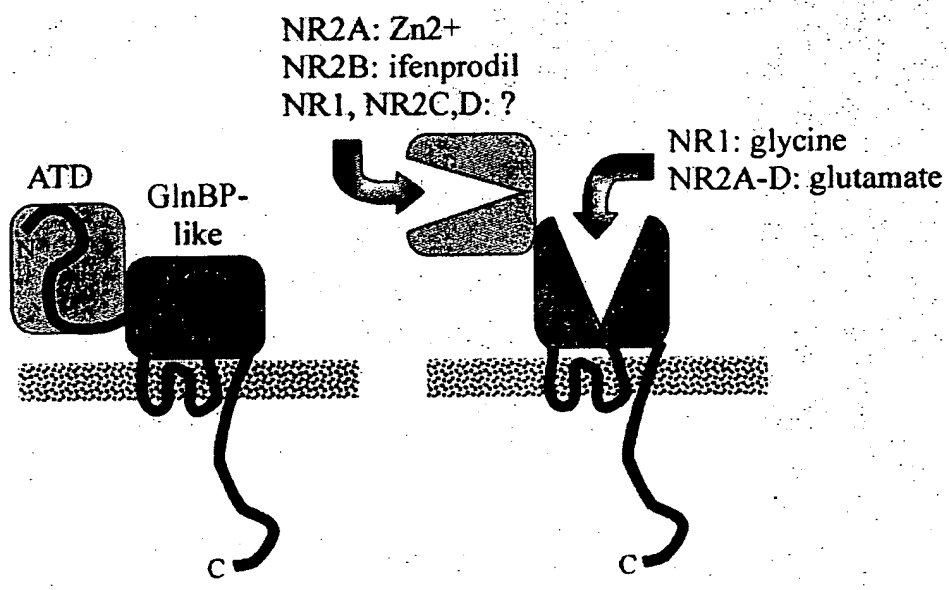


Fig. (2). Schematic representation of NMDA receptor.

calcium ions. At membrane potentials that correspond to the membrane potential of most neurons at rest (below –50 mV), extracellular magnesium ions block NMDA receptors, even when the receptor is activated by both agonists, glycine and glutamate [47]. However, due to the charged nature of Mg^{2+}, this block is voltage dependent and is readily reversed upon depolarization of the membrane. In the case of spinal nociceptive processing, depolarization is achieved by the same molecular signal that activates NMDA receptors: glutamate release from A- and C-fibers terminating in the spinal cord will open AMPA and KA channels and thereby depolarize the spinal cord neurons sufficiently to induce relief of Mg^{2+}-block of the co-localized NMDA receptors [48]. Because open NMDA channels are permeable not only to monovalent cations like Na^+ and K^+, but also to Ca^{2+}, this release from extracellular magnesium block will result in a transient and spatially restricted elevation of intracellular calcium levels. This in turn will trigger activation of calcium-dependent proteins localized within the affected area, and subsequent activation of other cellular processes, that can ultimately lead to cell death in the extreme of over-excitation [49]. As part of the regulation of glutamate receptors, released glutamate is removed from extracellular space by a glutamate transport system, which then allows the NMDA receptors to return to a resting state [50].

NMDA RECEPTORS AND CENTRAL SENSITIZATION

Chronic neuropathic pain is a result of pathological alterations in the central nervous system that persist long after the painful stimuli has been removed [51-53]. One of these types of pathological alterations is termed central sensitization and is a result of hyperexcitability of dorsal horn neurons [54]. Sensitization can occur after repeated stimuli of peripheral inputs, chemical stimulation or central pathological events [55]. Another related circuitry change is a specific phenomenon known as "wind-up", caused by repetitive C-fiber activation that results in abnormal firing of the dorsal horn neurons [56]. It is also hypothesized that spinal cord injuries result in changes of the circuitry and lead to central sensitization [57]. Furthermore, it is believed that central sensitization may underlie the neuropathological pain conditions of hyperalgesia (exaggerated nociceptive response to noxious stimuli) and allodynia (nociceptive responses to innocuous stimuli) [58].

It has been established that NMDA receptors are involved in central sensitization in the dorsal horn of spinal cord [59, 60, 61]. As an example, it has been demonstrated that NMDA antagonists can both block and reverse central sensitization [59]. Similarly, the phenomena of wind-up has been linked to NMDA receptors [62, 63]. For example, ketamine [60], MK-801 [64] and memantine [64] have all been reported to inhibit wind-up on dorsal horn neurons.

EVIDENCE OF GLUTAMATE AND GLUTAMATE RECEPTOR REGULATION IN NOCICEPTIVE PROCESSING

During nociceptive events, the number and subunit composition of glutamate receptors are altered from the resting state. For example, following the administration of Freund's complete adjuvant (FCA), the numbers of peripheral glutamate receptors in primary afferent neurons were increased following immunochemical staining [65]. It has likewise been observed that in the formalin pain model, differences in NMDA receptor expression are noticed. After administration of formalin (in the formalin pain model), the levels of NR2A expression are increased in the spinal cord while NR2C levels are decreased [66]. In a different study, spinal nerve lesion resulted in a decrease of NR2A receptors in the dorsal horn [67]. Another study has also implicated the NR1 subunit in central sensitization. Conditional knock-out mice of the NR1 NMDA subunit in the lumbar spinal cord had diminished nociception in the formalin pain model, but did not have alterations in normal pain thresholds to cold, heat or mechanical stimulation [68].

Along with changes in NMDA receptor populations, concentrations of glutamate are like-wise altered in animal pain models. In one study the concentration of peripheral glutamate increased in the ipsilateral paw, but not the contralateral paw following formalin injection [69]. In another study, concentrations of glutamate and aspartate increased in the ipsilateral side of the dorsal horn in a rat CCI model of neuropathic pain. Treatment of MK-801 led to a suppression of this excitatory amino acid increase [70]. Consistent with the link between elevated levels of glutamate in certain pain models, it has been observed that intrathecal L-glutamate can cause allodynia by itself [71].

NMDA ANTAGONISTS IN MODELS OF PAIN

The link between NMDA receptors and neuropathic pain has been well validated in animal models of pain. One early report of an NMDA antagonist in pain model was that of APV, (intrathecal administration), however these studies demonstrated side effects which complicated the analysis [72]. Since then, all of the various classes of NMDA antagonists have been studied in a variety of pain models and are summarized in this review.

A very common pain model that has been used to profile a wide-variety of structurally diverse NMDA antagonists is the formalin pain model [73, 74]. This model has two distinct phases of nociception that are believed to represent peripheral and centrally mediated pain. The early phase (EP) (transient) is thought to be a response to the peripheral nociception [75], whereas the late-phase (LP) is hypothesized to be the result of changes in the CNS function including central sensitization of dorsal horn neurons [76]. Formalin also causes excitation of C-fibers in a biphasic manner that is consistent with EP and LP observations in rat models of formalin induced pain [62]. Some have suggested that this model is similar to post-operative pain [77].

Another well studied pain model with respect to NMDA antagonists is the nerve injury or ligation model, of which several varieties are known, including the chronic constrictive injury model (CCI model) [78], the spinal nerve ligation model (Chung model) [79] and the partial sciatic nerve injury model [80]. Other pain models are included in this report, even though their link to human neuropathic pain or NMDA receptor activity has not been as well characterized. Studies in acute pain models (*e.g.* tail flick test) have also been included in some circumstances in order to provide the reader some comparison to the various models

used with NMDA antagonists. It is generally accepted that most NMDA antagonists do not work in these models [81], although there are some exceptions in the tables below. It has been reported that NMDA antagonist side effects can sometimes confound the results of certain acute pain models [81], and thus these results must be taken into the broader context of knowledge about NMDA antagonists and motor/CNS function side-effect profiles.

There have also been many reported studies in both acute pain models and chronic pain models on the effect of NMDA antagonists to potentiate low-doses of other classes of pain drugs such as opiates [82, 83]. It is not the purpose of this review to cover this large topic, however, some selected examples are discussed where direct comparisons could be made either to the compound or compounds of similar type in a relevant pain model.

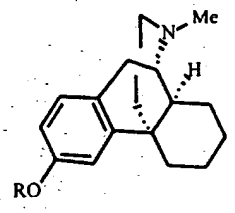
The underlying hypothesis and potential value of NMDA antagonists rests on the potential for blocking pathological pain mechanisms without severe impact to the normal physiological pain processes. Evidence for this hypothesis was largely driven by electrophysiological data indicating that NMDA antagonists can block wind-up without alteration of basal neuronal firing [84]. This hypothesis was reinforced by behavioral data supporting the fact that NMDA antagonists in general do not alter basal paw withdrawal latencies in the contralateral paw in models of neuropathic pain [85]. In this respect NMDA antagonists may be seen as "targeted therapy" and may have advantages over drugs which attenuate normal physiological pain in a broad sense.

The following sections of this review contain summaries of *in vitro* pharmacology, pain-related behavioral models, animal models of pain as well as human clinical trial results. Although this covers a large majority of known NMDA antagonists, it is not meant to be a comprehensive collection of all known studies. References and compounds were selected so as to provide both a breadth of diversity of compounds, a comparison to the most well-studied compounds, and a historical perspective of the development of individual classes of antagonists. The sections are subdivided into the four classes of antagonists.

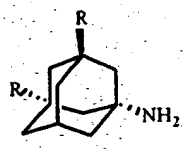
NON-COMPETITIVE ANTAGONISTS: CHANNEL BLOCKERS

The NMDA channel is characterized by a high affinity to PCP with a reported K_d value for PCP of 0.027 μM in rat brain synaptic membranes [86]. PCP is not considered a candidate for neuropathic pain treatments due to an obvious liability of psychotomimetic side effects and abuse potential. Therefore, the challenge in finding channel blockers with therapeutic utility is to identify compounds which lack PCP-like pharmacology. Chemical structures of the compounds addressed in this section are shown in Figure 3. Compounds reported to be channel blockers that have been tested in pain models are then summarized in Table 1.

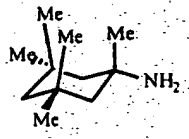
Dextromethorphan and related analogs are similar in structure to the opiods, but differ in that they possess the dextrorotary configuration. This stereochemical change results in a loss of significant opiod activities [87]. Clinically, dextromethorphan is currently used as an anti-tussive. Dextromethorphan shows modest selectivity for NR1A/NR2A over NR1A/NR2B (approx. 4-fold) [88]. A related compound, dextrorphan, is a more potent metabolic side product of dextromethorphan. For a detailed discussion of dextrorphan binding see Franklin and Murray [89].



Dextromethorphan, R = Me
Dextrorphan, R = H



Memantine, R=Me
Amantadine, R = H



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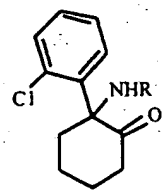
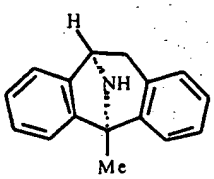


Ketamine, R = Me
Norketamine, R = H



MK-801
Dizocilpine

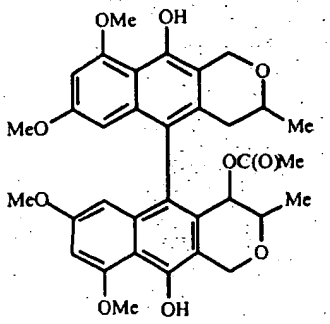


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Fig. (3). Non-Competitive NMDA Antagonists.

Table 1. Pain Data Associated with Non-Competitive NMDA Receptor Antagonists

Antagonist/*In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
Dextromethorphan IC_{50} = 2.5 μM [86][c]	Formalin	i.th.	r	(+/-)		[107]
	Chung Model	i.th.	r	(+)	(+)[d]	[107]
	Mechanical allodynia, spinal injury	i.p.	r	(+)	(+)	[108]
	Opiate enhancement		h	(-)		[109]
	Writhing, NSAID potentiation	i.p.	m	(+)		[110]
	Diabetic neuropathy	p.o.	h	(+)	(-)	[111]
	Postherpatic neuralgia	p.o.	h	(-)		[111]
	Oral surgery, post-operative pain	p.o.	h	(+)[e]	(-)[f]	[112]
	Heat-capsaicin model, volunteers	i.v.	h	(+)	(-)[g]	[113]
Dextrorphan IC_{50} = 0.68 μM [86][c]	Chung Model	i.th.	r	(+)	(+)[d]	[107]
	Formalin, LP	i.th.	r	(+/-)		[107]
	Formalin, LP	i. pl.	r	(+/-)[h]		[114]
	Thermal hyperalgesia, CCI	i.p.	r	(+)		[115]
	Mechanical hyperalgesia, CCI	i.p.	r	(-)		[115]
	Tail-flick- opiate potentiation, spinal injury	s.c.	r	(+)	(-)	[116]
Memantine K_i = 2.45 μM [97][i]	Chung Model	i.th.	r	(+)	(+)[d]	[107]
	Formalin, LP	i.th.	r	(+)	(+)[d]	[107]
	Formalin, EP	s.c.	m	(+)	(-)	[74]
	Formalin, LP	s.c.	m	(+)	(-)	[74]
	Formalin, facial pain, EP	i.p.	r	(+/-)		[117]
	Formalin, facial pain, LP	i.p.	r	(+)	(+)	[117]
	Formalin, LP	i. pl.	r	(+/-)		[114]
	Thermal hyperalgesia, CCI	i.p.	r	(+)	(+)	[118]
	Thermal hyperalgesia, carrageenan	s.c.	r	(+)	(+)	[119]
	Visceral pain, uterer distension	i.v.	r	(+)		[120]
	Thermal hypersensitivity, PGE2	i.p.	r	(-)		[121]
	Toe-pinch	i.v.	r	(+)[j]		[120]
	Writhing, NSAID potentiation	i.p.	m	(+)		[110]
	Diabetic neuropathy	p.o.	h	(-)		[111]
	Postherpatic neuralgia	p.o.	h	(-)		[111]
	Post-operative pain, amputations, nerve injuries		h	(-)		[122]
Amantadine K_i = 25.87 μM [97][i]	Surgical neuropathic pain, cancer patients	i.v.	h	(+)	(+)	[123]
	Neuropathic pain	i.v.	h	(+)[k]		[124]
	Diabetic neuropathy	i.v.	h	(+)		[125]

(Table 1) Contd....

Antagonist/*In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
MRZ 2/579 K_i = 1.47 μM [97][f]	Thermal hyperalgesia, carrageenan		r	(+)	(+)[f]	[96]
Ketamine IC_{50} = 0.46 μM [129][e]	Thermal hyperalgesia.	i.th.	r	(+)	(+)	[126]
	Pressure, opiate potentiation	s.c.	r	(+)		[127]
	Carrageenan, opiate potentiation	s.c.	r	(+)		[128]
	Tail flick	i.th.	m	(-)		[129]
	Chung Model	i.th.	r	(-)		[107]
	Thermal hyperalgesia, carrageenan	i.th.	r	(+)	(+)	[85]
	Formalin, EP	s.c.	m	(+)	(-)	[74]
	Formalin, LP	s.c.	m	(+)	(-)	[74]
	Formalin, LP	i. pl.	r	(+/-)		[114]
	Formalin, LP	p.o.	r	(+)	(+)[f]	[99]
	Mechanical allodynia, transgenic[j]	i.p.	m	(+)		[130]
	Visceral pain, uterer distension	i.v.	r	(+)		[120]
	Toe-pinch	i.v.	r	(-)		[120]
	Writhing test, NSAID potentiation	i.p.	m	(+)		[110]
	Thermal hypersensitivity, PGE2	i.p.	r	(-)		[121]
	Fibromyalgia patients[m]	i.v.	h	(+)		[131]
	Secondary hyperalgesia/ "wind-up" like pain	i.v.	h	(+)	(-)	[132-134]
	Neuropathic pain	i.v.	h	(+)	(-)	[135]
	Heterogeneous painful neuropathies	i.v.	h	(+)	(-)	[104]
	Stump /phantom limb-pain -amputees	i.v.	h	(+/-)	(-)	[136]
	Experimental ischemic pain /	i.v.	h	(+)	(+)	[137]
	Postoperative pain, oral surgery	i.v.	h	(+)	(+)	[137]
MK-801 IC_{50} = 0.015 μM [129][e]	Tail flick test	i.th.	m	(-)		[129]
	Thermal hyperalgesia, CCI	i.th.	r	(+)	(+)	[126]
	Thermal hyperalgesia, CCI	i.p.	r	(+)		[138, 139]
	Self-mutilation, nerve injury	i.th.	r	(+)		[140]
	Chung model	i.th.	r	(-)		[107]
	Formalin, LP	i.th.	r	(+)	(+)[d]	[107]
	Formalin, EP	s.c.	m	(+)	(-)[d]	[74]
	Formalin, LP	s.c.	m	(+)	(-)[d]	[74]
	Mechanical allodynia, nerve injury	i.p.	r	(+)	(-)	[141]
	Mechanical hyperalgesia, carrageenan	i.p.	r	(+)	(-)	[141]
	Thermal hyperalgesia, carrageenan	i.th.	r	(+)	(+)	[85]

(Table 1) Contd....

Antagonist/*In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
	Thermal hyperalgesia, nerve injury	i.th.	r	(+)	(+)	[142]
	Mechanical allodynia, spinal cord injury	i.p.	r	(+)	(-)	[108]
	Mechanical hyperalgesia, post-operative pain	i. th.	r	(-)		[143]
	Mechanical hyperalgesia (periph.), FCA	i. pl.	r	(+)		[144]
	Allodynia, opiate induced	s.c.	r	(+)		[145]
	Thermal hypersensitivity, PGE2	i.p.	r	(-)		[121]
Mg^{2+}	CCI	i.p	r	(+)	(+)	[146]
	Formalin, LP	i.th.	r	(+)	(+)	[147]
	Heterogeneous painful neuropathies, spontaneous pain, allodynia	i.v.	h	(-)		[104]
ES-242-1 IC_{50} = 0.116 μM [105,106][w]	Formalin, EP	i.th.	r	(+)		[148]
	Formalin, LP	i.th.	r	(+)		[148]

[a] Route of administration and species tested: i.th. = intrathecal, i.p. = intraperitoneal, i.m. = intramuscular, i. pl. = intraplantar, s.c. = subcutaneously, i.v. = intravenously, p.o. = oral,; r = rat, m = mouse, h = human. [b] Result : (+) = postive result, (-) = negative result, (+/-) = limited efficacy or incomplete attentuation. Side effect separation: (+) = no noticeable effects in assay, (-) = noticeable effects, no value in column indicates that the side effect profile was not reported. [c] Versus [^{3}H] PCP. [d] Separation observed in separate motor side effect assay. [e] 48 hours post surgery, but not at 6 hours. [f] Adverse effects seen above placebo. [g] Drowsiness reported after infusion. [h] Lifting behaviors and righting reflexes were attenuated, but not flinching. [i] Versus [^{3}H] MK-801. [j] Authors claim positive effect may be due to nonspecific cardiovascular effects. [k] Tested in only three patients. [l] Positive (30 mg/kg), but side effects evident at 60 mg/kg p.o. [m] Muscle pain, temporal summation, and referred pain. [n] Versus [^{3}H] TCP. [o] Rat brain receptors in Xenopus oocytes.

Memantine is a clinically available (Parkinson's disease, and more recently Alzheimer's disease) NMDA antagonist with a low side effect profile [90, 91]. It is reported to have no selectivity for NR1A/NR2A over NR1A/NR2B [88]. Memantine is considered unique among the channel-blockers because it displays fast blocking kinetics and possesses a strong voltage-dependence of the block. It is surmised that these properties may contribute to the lack of PCP-like side effects [92, 93]. A recent report has coined memantine as being part of a class of "pathologically activated therapeutics"(PAT), thus describing its relatively good side-effect profile under non-stress conditions [94].

In electrophysiology studies, memantine (*s.c.* admin.) reduced neuronal wind-up in a Chung model with little effect on sham-operated rats (in contrast to MK-801 and ketamine which reduced wind-up in both animal models) [91]. It has demonstrated a reduced activity on spinal cord neurons in a rat arthritis model in response to noxious and innocuous stimuli (*i.v.* admin.) [95]. With respect to side effect profiles, it does not show activity in pre-pulse inhibition of the acoustic startle reflex (PPI), a model of psychotomimetic side effects. This result contrasts with MK-801 and PCP which do show adverse events in this model [96]. Other related analogs to memantine are amantadine and MRZ 2/579. Amantadine is clinically used as an anti-viral compound and has very weak channel blocking properties. MRZ 2/579, which is similar in structure to memantine, also displays fast blocking kinetics (K_{on} = 10.67 ± 0.09 x 10^4 M^{-1} s^{-1}, K_{off} 0.199 +/- 0.02 s^{-1}, K_d = K_{off}/K_{on} = 1.87 μM.) [97]. Again, the authors argue that low-affinity channel blockers such as memantine and MRZ 2/579 have a clear advantage over high affinity channel blockers such as MK-801 with respect to an improved side effect profile. Alternatively, this could be due to other factors including blocking kinetics, voltage dependence or subtype-selectivity [96].

Ketamine is another member of the non-competitive NMDA channel blocking antagonists, but also possesses activity at other receptors including opiod, monoaminergic, muscarinic, and voltage-sensitive Ca^{2+} channels [98]. Intravenous administration to rats during the second phase of formalin prevented dorsal horn nociceptive neuron firing in a dose-related manner, with only a small effect in the first phase [73]. Ketamine can be rapidly metabolized to norketamine, which has equipotent activities to ketamine itself. Thus, the authors conclude that norketamine could be responsible for part of the nociception observed with ketamine [99].

Ketamine is generally not used in the clinic because it induces a trance-like cataleptic state [100]. A more comprehensive report on ketamine clinical trial data has been published, and the conclusion from the data (53 clinical trials) is that the role of ketamine in perioperative pain is unclear [101].

MK-801 is a potent channel-blocker with no selectivity for NR1A/NR2A over NR1A/NR2B [88]. When MK-801 was iontophoretically administered to CCI rats with thermal and mechanical stimulated hyperalgesia, it blocked noxious

evoked response in WDR neurons, but not baseline hyperactivity. It is suggested by the authors that the baseline hyperactivity is likely related to spontaneous pain [102]. Administration (*i.v.*) to rats during the second phase of the formalin pain assay prevented dorsal horn nociceptive neuron firing in a dose-related manner, with only a small effect in the first phase. Pre-treatment before formalin also gave dose-related inhibition with little effect on the first phase [73]. Similarly when dosed intravenously to rats, MK-801 blocked noxious-stimuli evoked activity in CCI rats in ventroposterolateral neurons as measured by electrophysiology. However, it also blocked this activity in normal rats in contrast to the glycine-site antagonist GV-196771A [103]. Pain studies for MK-801 are summarized in Table 1.

Since magnesium serves as an endogenous channel-blocker, magnesium salts have been studied as potential pain therapeutics. At least one clinical trial has been reported, however, magnesium salts were not efficacious in this study [104].

Finally, a microbial natural product, ES-242-1, is reported to interact with NMDA receptors, predominately as a channel blocker. However, ES-242-1 also has competitive activity at the glutamate binding site (IC_{50} = 1.1 μM *vs.* [^{3}H] CPP) [105, 106]. The structure appears to be quite unique in that it does not possess a basic amine. Very limited studies have been done in pain studies with this compound, however these results are shown in Table 1.

COMPETITIVE GLUTAMATE SITE ANTAGONISTS

Most of the glutamate-competitive antagonists that are reported in Figure 4 belong to the phosphono-amino-acid class of compounds. These compounds were originally developed for treatment of stroke and stroke related diseases, but have so far not proved clinically useful for these applications. One reason for this is a poor therapeutic margin and the large doses required for optimal brain penetration [149]. Subsequent studies in animal pain models have demonstrated activity, with however, the expected side effects being observed. Although there are some exceptions when comparing different pain models, most of these compounds have motor side effects with a narrow therapeutic window. In contrast to the channel blockers (non-competitive), many of the glutamate-competitive antagonists are active in both early phase and late phase formalin. In some cases these compounds are also active in tonic (phasic) pain models. In the various studies reported these compounds are typically administered intrathecally, suggesting that blood-brain barrier (BBB) penetration may be difficult for some of these compounds. However, some examples of systemic administration are included below. Two naturally occurring peptides are included in this review Con-G and Con-T. Although these may not represent small molecule therapeutics, it is useful to highlight the structural diversity in this class of antagonists.

CPP is a potent and competitive antagonist. Intrathecal administration to rats prevented dose-related dorsal horn nociceptive neuron firing in the second phase of formalin assay with only a small observable effect in the first phase [73]. Very limited studies have been reported in pain assays, most of which resulted in the observation of motor side effects at therapeutic doses (Table 2). Similar to CPP, 2-amino-5-phosphonovalerate (APV or AP-5) dose-dependently prevented dorsal horn nociceptive neuron firing in the second phase of the formalin assay, with only a small effect in the first phase [73]. APV has also been reported to reduce electrically stimulated wind-up in rats [162]. Injection into the hippocampal dentate gyrus, before and after formalin first phase, led to reduction of nociceptive behaviors. This result led the authors to conclude that pain related behaviors associated with the NMDA receptor could be involved in the hippocampal region [163]. When APV was co-administered with glycine an increase in the antinociceptive activity across a range of doses was observed in the mouse formalin model. The effect was reversed with the addition of 7-CKA [164]. There was no synergistic effect with morphine in thermal hot plate test (*i.th.* administration, rats) [165].

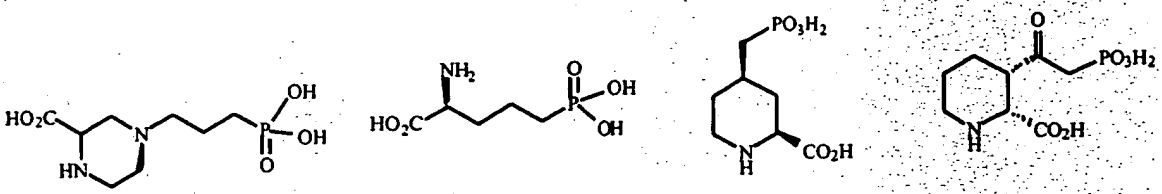
Conformationally restricted analogs of APV, such as CGS-19755 (Selfotel), MDL 100-925, LY235959, CGP 37849 and CGP 39551 have also been studied in pain models. The results are reported in Table 2. It may be expected that these compounds suffer from poor brain penetration due to the charged nature. This has been surmised for LY235959 which was less potent when administered subcutaneously as compared to an intrathecal route [158].

Perzinfotel (EAA-090) is also a high-affinity competitive antagonist for glutamate. The compound demonstrated a superior side effect profile over other NMDA antagonists of similar kind in PGE2 and capsaicin pain models. Of interest is the fact that some NMDA antagonists (ketamine, memantine, ifenprodil and others) did not show activity in this model, thus perhaps indicating a novel mechanism of action for perzinfotel [121].

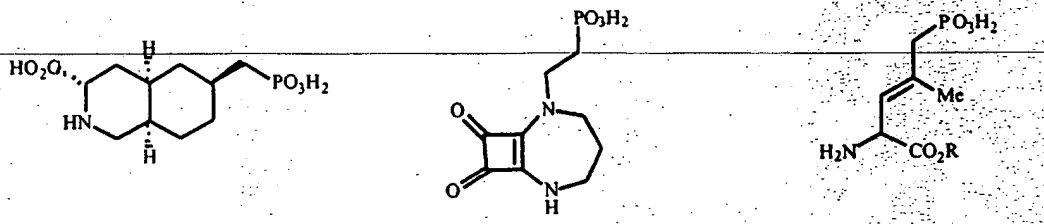
Conantokin G (CGX-1007 or Con-G) is a 17 amino-acid peptide isolated from cone snail venom of the genus *Conus*, and is reported to selectively bind to the NR2B subunit [161]. It has an IC_{50} of 0.48 μM on the NR2B-component of NMDA-evoked currents from cultured cortical neurons. No response was seen above 10 μM for the other subtypes [161]. Conantokin G exists in a helical form that is stabilized by the presence of divalent cations such as Ca^{2+} and Zn^{2+} [166, 167]. However, there does not appear to be a clear relationship between this property and the observed binding activity in the absence or presence of these metals [166, 167]. The mechanism of action of the conantokins has been challenging to understand. Although it is likely that these are competitive antagonists [166, 167, 168] others have suggested a role in the allosteric polyamine modulatory site [169, 170]. For a recent review the reader is referred to Castellino [171]. Conantokin T is an analog of conantokin G. This compound binds to both NR2A and NR2B [161, 171].

COMPETITIVE GLYCINE SITE ANTAGONISTS

As discussed in the introduction, occupancy of the glycine site is required for activation of NMDA receptors. Among the lines of evidence in this regard is that glycine has been reported to enhance the affinity of MK-801 in washed rat cortical membranes [172]. Further evidence is gained by studies that have demonstrated an enhancement of NMDA-mediated post-synaptic potentials in neocortical slices



(±) CPP APV, AP5 CGS 19755 Selfotel MDL 100,925



LY 235959 Perzinfotel = EAA-090 CGP 37849, R = H CGP 39551, R = Me

NH_2-GEγγLQγNQγLIRγKSN-$CONH_2$

Conantokin G

γ = γ-carboxyglutamic acid

NH_2-GEγγYQKMLγNLRγAEVKKNA-$CONH_2$

Conantokin T

Fig. (4). Competitive NMDA Antagonists

Table 2. Pain Data Associated with Competitive Glutamate Site Antagonists

Antagonist/*In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
CPP IC_{50} = 0.32 μM [150][c]	Thermal hyperalgesia, carrageenan	i.th.	r	(+)	(+)	[85]
	Formalin, LP	s.c.	m	(+)	(-)	[74]
	Tail flick	i. th.	r	(+)	(+)[d]	[152]
	Hot plate	i. th.	r	(+)	(+)[d]	[152]
	Formalin, EP	i. th.	r	(+)	(+)[d]	[152]
	Formalin, LP	i. th.	r	(+)	(+)[d]	[152]
	Thermal hypersensitivity, PGE2	i.p.	r	(+)		[121]
APV K_i = 0.35 μM [153][e]	Formalin, LP	i.th.	r	(+)	(-)	[151]
	Mechanical allodynia, spinal injury	i.th.	r	(+)	(+)	[57]
	Thermal allodynia spinal injury	i.th.	r	(-)		[57]
	Chung model	i.th.	r	(+)	(+)[f]	[107]
	Formalin, LP	i.th.	r	(+)	(+)[f]	[107]
	Thermal hyperalgesia, CCI	i.th.	r	(+)	(+)	[126]
	Self-mutilation, nerve injury	i.th.	r	(+)		[140]
	Mechanical hyperalgesia, incision	i.th.	r	(-)		[143]
	Thermal hyperalgesia, nerve injury	i.th.	r	(-)		[142]

(Table 2) Contd....

Antagonist/*In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
	Thermal hyperalgesia, carrageenan	i.th.	r	(+)	(+)	[85]
	Muscle hyperalgesia , FCA	i.m.	r	(+)		[154]
CGS-19755 K_i =0.040 μM [155][e]	Thermal hypersensitivity, PGE2	i.p.	r	(-)		[121]
	Formalin, EP	s.c.	r	(-)		[156]
	Formalin, EP	s.c.	m	(+)	(-)[g]	[74]
	Formalin, LP	s.c.	m	(+)	(-)[g]	[74]
	Formalin, LP	s.c.	r	(+)	(+)[d]	[156]
	Mechanical allodynia, spinal cord injury	i.p.	r	(+)	(-)	[108]
MDL 100,925 K_i = 0.064 μM [155][e]	Hot plate	i.th.	m	(+)		[155]
	Formalin, EP	i. th.	m	(+)		[155]
	Formalin, LP	i. th.	m	(+)		[155]
LY235959 K_i = 0.025 μM [157][e]	Thermal hyperalgesia, NMDA	i. th.	r	(+)	(+)	[158]
	Formalin, LP	i. th.	r	(+)	(+)	[158]
	Formalin, LP	s. c.	r	(+)	(-)	[158]
CGP 37849 IC_{50} = 0.025 μM [150][c]	Formalin, EP	s.c.	m	(+)	(-)[g]	[74]
	Formalin, LP	s.c.	m	(+)	(-)[g]	[74]
	Tail-flick, heat or pressure	s.c.	m	(-)		[74]
CGP 39551 IC_{50} = 0.025 μM [150][e]	Formalin, EP	s.c.	m	(+)	(-)[g]	[74]
	Formalin, LP	s.c.	m	(+)	(-)[g]	[74]
	Tail-flick, heat or pressure	s.c.	m	(-)		[74]
Perzinfotel (EAA-090): IC50= 0.030 μM [159][h]	Thermal hypersensitivity, PGE2	i.p.	r	(+)		[121]
	Thermal hypersensitivity, PGE2	p.o.	r	(+)		[121]
	Thermal hypersensitivity, capsacin	i. p.	r	(+)		[121]
	Thermal hypersensitivity, capsacin	p.o.	r	(+)		[121]
Conantokin G IC_{50} =0.48 μM (electrophys) [161][i]	Formalin, EP	i. th.	m	(-)		[160]
	Formalin, LP	i. th.	m	(+)	(+)	[160]
	Thermal allodynia, nerve injury	i. th.	m	(+)		[160]
	Mechanical allodynia, nerve injury	i. th.	m	(+)		[160]
	Thermal allodynia, FCA	i. th.	m	(+)		[160]
	Mechanical allodynia, FCA	i. th.	m	(+)		[160]
Conantokin T	Formalin, EP	i. th.	m	(-)		[160]
	Formalin, LP	i. th.	m	(+)	(+)	[160]

[a] Route of administration and species tested: i.th. = intrathecal, i.p. = intraperitoneal, i.m. = intramuscular, i. pl. = intraplantar, s.c. = subcutaneously, i.v. = intravenously, p.o. = oral,; r = rat, m = mouse, h = human. [b] Result : (+) = postive result, (-) = negative result, (+/-) = limited efficacy or incomplete attentuation. Side effect separation (+) = no noticeable effects in assay, (-) = noticeable effects, no value indicates that side effect profile was not reported. [c] Versus [^{3}H] L-glutamate. [d] Narrow window versus side effects. [e] Versus [^{3}H] CGS 19755. [f] Side effects seen at higher doses. [g] Therapeutic doses tested in motor function assay. [h] Versus [^{3}H] CPP. [i] Determined in NMDA evoked currents in competition with CPP and other classes of NMDA antagonists.

following glycine application [173]. It is generally thought that glycine site antagonists may possess a better side effect profile over competitive antagonists and channel blockers [174].

Similar to competitive antagonists at the glutamate site, one difficult challenge surrounding glycine-site antagonists has been to identify compounds with high levels of brain penetration. This is most likely due to high plasma protein binding [175] and perhaps the charged nature of these compounds at physiological pH ranges. Interestingly, glycine itself has been shown to prevent mechanical hyperalgesia in rat models of neuropathic pain [176]. This would seem to contradict the theory that glycine-site antagonists are useful in neuropathic pain. However, these effects are proposed to be due to activation of the inhibitory glycine receptors (strychnine sensitive), which would decrease excitability of spinal neurons. A summary of known pain activities of glycine-site antagonists is described below. Some allosteric partial agonists are known, such as D-serine and D-cycloserine, and are reported in this review since they have been studied in pain models. Structures of these compounds are illustrated in Figure **5** and Figure **6**.

The early studies on indole carboxylic acids led to the development of some very potent glycine-site antagonists. The two most advanced indole carboxylic acids MDL 29,951 and GV196771A are reported in Table **3**. Dosed intravenously to rats, GV196771A blocked noxious-stimuli evoked activity in CCI rats in ventroposterolateral neurons as measured by electrophysiology. It did not alter this activity in normal rats, in contrast to MK-801 which blocked the activity in both sets of animals. The authors suggest that these compounds may block nociceptive signals in the thalamus [103]. GV196771A has also been reported to reduce the morphine tolerance in early phase and late phase formalin [198].

Another class of compounds, the quinoxaline-2,3-diones, have also been exploited as glycine-site antagonists. DCQX (6,7-dichloroquinoxaline-2,3-dione) is one of the first generation quinoxaline-2,3-diones, and has a reported IC_{50} *vs* [^{3}H] DPCQ of 0.13 μM [183]. It is important to note that some of these compounds do show activity against non-NMDA receptors (DCQX K_b = 4.8 μM in non-NMDA receptors, expressed receptors in oocytes [180]). Another example is ACEA-1011 with a reported affinity towards NMDA receptors expressed in Xenopus oocytes between 0.4 to 0.8 μM, while AMPA receptor binding activity was 8 μM in the same study [182].

While ACEA-1021 (Licostinel) is a much more potent analog in this series, it also possesses activity in non-NMDA receptors. ACEA-1021 has a reported affinity of K_b = 0.0059 μM in NMDA receptors [183] and a K_b between 1.5 to 3.3 μM in cultured rat brain neurons for AMPA receptors [199]. It is surmised that some of the phasic pain activity may be due to AMPA activity [199]. Another member of this series, ACEA-1328, also has activity at AMPA receptors (K_b of 0.039 μM at NMDA and 3.1 μM at AMPA receptors) [129].

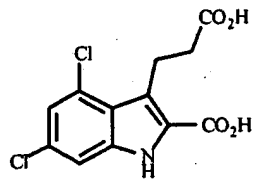
D-Serine is an endogenous co-agonist with a weak reported affinity for [^{3}H] glycine at rat brain membranes of 0.67 μM [186]. However, mice deficient in D-amino-acid-oxidase (DAAO – an enzyme that deoxygenates D-amino acids) have an exaggerated response in late stage formalin. This would suggest that high levels of D-serine could contribute to nociception. The authors suggest that DAAO plays an important neuromodulatory role in regulating D-serine levels [200].

Analogs of D-serine have been explored such as D-cycloserine, a partial agonist at the glycine site. It has a reported intrinsic activity of 57% (*vs.* glycine) in cultured hippocampal neurons [97]. Related to D-cycloserine, R(+)-HA966 is another partial agonist/antagonist at the glycine site, with a reported IC_{50} *vs.* [^{3}H] glycine of 12.5 μM in rat cortical membranes. It was also found to have a K_i = 2.5 μM versus glycine potentiated NMDA responses [188] with an intrinsic activity of 13% in cultured hippocampal neurons [194]. Administration of R(+)-HA966 reduced thermal hyperalgesia before injury and after injury in the CCI rat model (i.th.) [189]. A final related compound, L-687,414, is a low efficacy partial agonist which acts as a functional antagonist at the glycine site [193]. The reported IC_{50} *vs.* [^{3}H] glycine was 1.4 μM in rat cortical membranes. The measured K_i was equal to 0.65 μM *vs.* glycine-potentiated NMDA responses [188]. Reports of pain studies are captured in Table **3**.

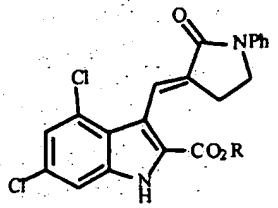
Another class of glycine antagonists evolved from kynurenic acid. Kynurenic acid is a metabolite of tryptophan and may act as an endogenous ligand at the glycine site of the NMDA receptor [201]. It is a weak antagonist with a reported IC_{50} of 41 μM *vs.* glycine in rat cortical membranes [202] and a reported IC_{50} of 7.9 μM *vs.* [^{3}H] MDL 105,519 [194]. Further potency was achieved with the introduction of a 7-chlorine substituent to give 7-chlorokynurenic acid. This compound is a more potent analog of kynurenic acid with a reported IC_{50} of 5.2 μM versus [^{3}H] glycine [202], and an IC_{50} of 0.320 μM [175] for displacement of [^{3}H]L-689,560 from rat cortical membranes. It is reported to have selectivity for NR1A/NR2A over NR1A/NR2B (>10-fold) [88]. Intrathecal administration to rats reduced wind-up after repetitive C-fiber stimulation. It also reduced neuronal responses in the formalin test [203].

DPCQ (or 5,7-DCK = 5,7-dichloro-2,4-dihydroxy-3-phenyl-quinoline dione) is a compound of the same family as 7-CKA. Of interest to the emerging data surrounding peripheral effects of NMDA antagonists, DPCQ was studied as a compound with limited CNS exposure as compared to L-701,324 (a glycine antagonist with higher CNS exposure). Both compounds were active in late-stage formalin, CCI and spinal nerve ligation models, but only L-701,324 worked to block iontophoretic adminstration of NMDA [204]. The conclusion reached by the authors is that peripheral NMDA receptors may play an important role in neuropathic pain mechanisms. L-701,324 is a highly selective and potent antagonist, which is reported to have good oral bioavailability and brain penetration [205].

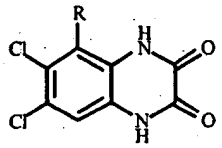
At least two series have been reported which mimic the kynurenic acid portion using a pyridazine-quinolinedione. The quinolinedione portion is acidic and is a suitable mimetic for the carboxylic acid functionality. MRZ 2/576 is reported to be a short-acting compound with rapid entry into the brain but with short duration [194]. A similar compound,



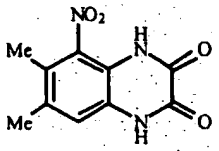
MDL 29,951



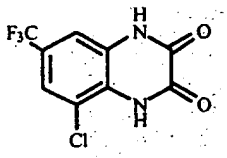
GV-196771, R = H
GV-196771A, R = Na^+



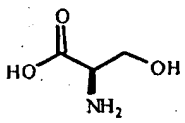
DCQX, R = H
ACEA-1021 (Licostinel)
R = NO_2



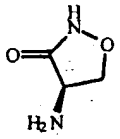
ACEA-1328



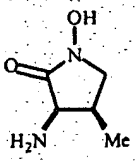
ACEA-1011



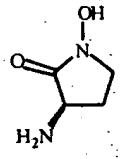
D-serine



D-cycloserine

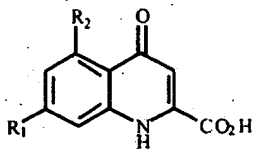


L-687-414

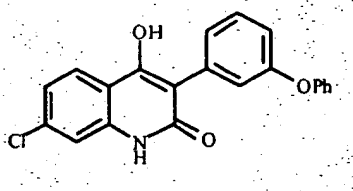


(+)-HA-966

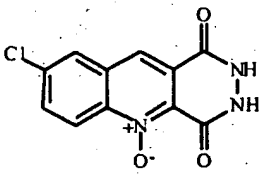
Fig. (5). Glycine-Site Antagonists and Partial Agonists.



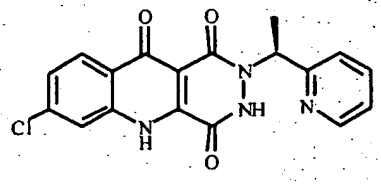
Kynurenic acid, R = H
7-CKA, R1 = Cl
DCPQ or 5,7-DCK R1 = R2 = Cl



MDL 701, 324



MRZ 2/576



AZ-2-PQT

Fig. (6). Glycine-Site Antagonists.

Table 3. Pain Data Associated with Allosteric Glycine Site Antagonists

Antagonist/ *In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
MDL 29,951 K_i = 0.14 μM [177][c]	Tail-flick, heat or pressure	i.p.	m	(-)		[74]
	Formalin, LP	i.p.	m	(+)	(+)	[74]
GV196771A K_i = 0.027 μM [178][c]	Formalin, EP	p.o.	m	(-)		[103]
	Formalin, LP	p.o.	m	(+)	(+)[d]	[103]
	Thermal hyperalgesia; CCI	p.o.	r	(+)	(+)[d]	[103]
	Mechanical hyperalgesia; CCI	p.o.	r	(+)	(+)[d]	[103]
	Chronic neuropathic pain	p.o.	h	(-)		[179]
DCQX Kb = 0.38 mM [180]e	Formalin, LP	i. th.	r	(-)		[151]
ACEA-1011 K_b= 0.4 to 0.8 μM [182][c]	Formalin, LP	i.p.	m	(+)	(+)	[181]
	Formalin, EP[e]	i.p.	m	(+)		[77]
	Formalin, LP[e]	i.p.	m	(+/-)[f]		[77]
ACEA-1021 K_b = 0.0059 μM [183][c]	Tail flick	i. th.	m	(+)		[129]
	Formalin	i.th.	m	(+)		[129]
	Formalin, opiate attenuation	i.th.	r	(+)	(-)[g]	[184]
ACEA-1328 K_b = 0.039 μM [129][c]	Tail flick	i. th.	m	(+)		[129]
	Tail flick, κ-opiod attenuation	i. p.	m	(+)		[185]
D-serine IC_{50} = 0.354 μM [186][c]	Formalin, κ,μ-opiod attenuation	i.c.v.	r	(+)		[187]
D-cycloserine IC_{50} = 7.37 μM [186][c]	Tail flick	s.c.	m	(-)		[74]
	Formalin, EP	s.c.	m	(-)		[74]
	Formalin, LP	s.c.	m	(+)	(+)[h]	[74]
R(+)-HA-966 IC_{50} = 12.5 μM [188][c]	Tail flick	i. th.	m	(-)		[74]
	Formalin, EP	s.c.	m	(-)		[74]
	Formalin, EP	s.c.	r	(-)		[156]
	Formalin, LP	s.c.	m	(+)	(+)[h]	[74]
	Formalin. LP	s.c.	r	(+)	(+)[h]	[156]
	Thermal hyperalgesia, CCI	i.th.	r	(+)		[189]
	Mechanical allodynia, opiod attenuation	s.c	r	(+)	(+)	[190]
	Mechanical allodynia, nerve injury- opiate attenuation	s.c.	r	(+)	(+)[h]	[191]
	Thermal allodynia, nerve injury - opiate attenuation	s.c.	r	(+)	(+)[h]	[191]
	Formalin, EP- attenuation of NK1 antagonist	s.c.	m	(+)	(+)[i]	[192]
L-687,414 IC_{50} = 1.4 μM[188][c]	Tail flick	s.c.	m	(-)		[74]
	Formalin, EP	s.c.	m	(-)		[74]
	Formalin, LP	s.c.	r	(+)	(+)[h]	[74]
	Mechanical hyperalgesia, carrageenan	i.p.		(+)	(-)[j]	[193]
	Mechanical allodynia, nerve injury	i.p.	r	(+)	(+)[h]	[141]

(Table 3) Contd....

Antagonist/ *In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
Kynurenic acid IC_{50} = 7.9 μM [194][f]	Thermal hyperalgesia, CCI	i.th.	r	(-)		[126]
	Thermal hyperalgesia, carrageenan	i.th.	r	(+)	(-)	[195]
	Thermal hyperalgesia, carrageenan--μ-opiod attenuation	i.th.	r	(+)	(+)	[195]
7-CKA IC_{50} = 0.32 μM [175][m]	Thermal hyperalgesia, carrageenan	i.th.	r	(+)	(+)	[85]
	Formalin, LP	i.th.	r	(-)		[151]
DPCQ or 5,7-DCK IC_{50} = 0.064 μM [175][m]	Formalin, EP	i.p.	m	(-)		[74]
	Formalin, LP	i.p	m	(+)		[74]
	Tail-flick, heat or pressure	i.p.	m	(-)		[74]
L-701,324 IC_{50} = 0.002 μM [193][m]	Mechanical hyperalgesia, carrageenan	i.p.		(+)	(+)	[193]
	Mechanical hyperalgesia, carrageenan	i.p.	r	(+)	(-)	[141]
	Mechanical allodynia, nerve injury	i.p.	r	(+)	(-)	[141]
	Thermal hypersensitivity, PGE2	i.p.	r	(+)		[121]
MRZ 2/576 IC_{50} = 0.100 μM [194][f]	Tail flick, opiate attenuation	i. p.	m	(+)[j]		[196]
	Uterer distension, reflex pressor response	i.v.	r	(+)		[120]
	Toe pinch	i.v.	r	(-)		[120]
AZ-2-PQT *Ki* = 0.207 μM [197][f]	Thermal hyperalgesia, CCI	p.o.	r	(+)	(+)[k]	[197][n]
	Formalin, LP	p.o.	r	(+)	(+)[k]	[197][n]

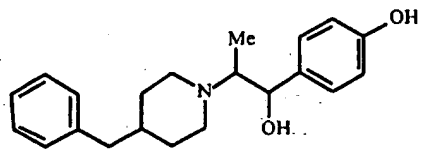
[a] Route of administration and species tested: i.th. = intrathecal, i.p. = intraperitoneal, i.m. = intramuscular, i. pl. = intraplantar, s.c. = subcutaneously, i.v. = intravenously, p.o. = oral,; r = rat, m = mouse, h = human. [b] Result : (+) = postive result, (-) = negative result, (+/-) = limited efficacy or incomplete attenuation. Side effect separation (+) = no noticeable effects in assay, (-) = noticeable effects, no value = not reported. [c] Versus [^{3}H] glycine. [d] Based on references cited therein. [e] Affinity against expressed receptors in Xenopus oocytes. [f] HA and LA MICE, in late-phase formalin the activity was seen in HA but not LA mice. [g] Side effects seen at maximal useable dose. [h] Therapuetic margin observed between *in vivo* pain assay and motor function assay. [i] Did not enhance the ataxia of NK1 antagonist. [j] Mild ataxia seen at MED. [k] Authors report only a narrow window versus side effects. [l] Versus [^{3}H] MDL 105,519. [m] Versus [^{3}H] L-689, 560. [n] *In vitro* data is reported in reference [197]. Pain data is unpublished, but previously reported: Brown, D. G.; Bare, T. M.; Urbanek, R. A.; McLaren, F. M.; Horchler, C. L.; Murphy, M.; Steelman, G. B.; Empfield, J. R.; Forst, J. M.; Herzog, K. J.; Xiao, W.; Dyroff, M. C.; Lee, C. M. C.; Trivedi, S.; Neilson, K. L.; Keith, R. A. 7-Chloro-2,3-Dihydro-2-[1-(pyridinyl)alkyl]-pyridazino[4,5-b]quinoline-1,4,10(5H)-triones as NMDA Glycine-Site Antagonists with Antinociceptive Activity. *Abstracts of Papers, MEDI-169, 227th ACS National Meeting*. Anaheim, CA, United States, March 28-April 1, 2004.

AZ-2-PQT, is a potent and selective full antagonist at the glycine site [197]. The compound demonstrated good oral bioavailability and oral activity in two distinct pain models. Pain data from these compounds are reported in Table 3.

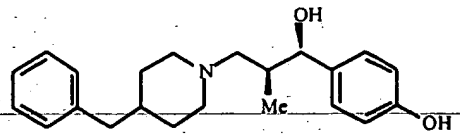
POLYAMINE SITE/IFENPRODIL SITE ANTAGONISTS

Both spermine and spermidine are known to modulate activity at the NMDA receptor. Polyamines of this type tend to have biphasic responses. Depending on the concentration and the structure of the polyamine, they may exert both agonist and antagonist modulation [206]. It has been reported that ifenprodil and other compounds of similar nature are non-competitive antagonists of the polyamine site [207]. Polyamine site antagonists related to ifenprodil tend to be selective for the NR2B subtype. This is observed within the heterogeneous assembly of the various subtypes, as different constructs of NR1/NR2A-D possess differing responses to polyamines. The NR2B subtype has been studied due to the potential for fewer and milder side-effects as compared to those associated with competitive antagonists and channel blockers. This is hypothesized based on the distribution of NR2B receptors as compared to other subtypes. In rat brain, NR2B is exclusively located in the forebrain and not the cerebellum [208]. It is also found to be located predominately in the dorsal horn of the spinal cord [141]. It is important to note that this field remains an area of active research and, as of yet, a clear understanding of the roles of subtype populations in neuropathic pain events has not been established. It is apparent however, that NR2B subtype selective antagonists do appear to have fewer side effects than competitive and non-competitive antagonists in animal models of neuropathic pain. It should also be noted that mice over-expressing NR2B receptors in the forebrain show greater mechanical allodynia after formalin or CFA compared to wild type. No changes were observed for acute pain [209]. For a review of NR2B pharmacology see Loftis [210].

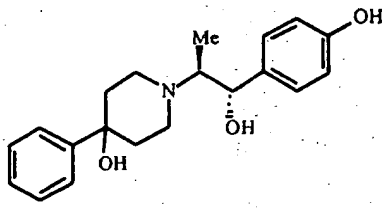
Ifenprodil is a potent NR2B selective antagonist that binds at the polyamine site [211, 212]. Ifenprodil demons-



Ifenprodil



Ro25-6981



CP-101,606

Fig. (7). Polyamine/Ifenprodil Site Antagonists

trated activity in carrageenan-induced mechanical nociception (i.p., rats), however did not demonstrate NMDA antagonism in spinal cord using electrophysiological studies (i.v.). These results suggest a supraspinal site of action [64]. (+/-)Ro 25-6981 is a close analog to ifenprodil, and is reported to have measured antinociceptive activity (Table 4).

Similar in structure to both ifenprodil and Ro 25-6981, CP-101,606 has also been studied in pain models. Even though it is a compound with a relatively short half-life ($t_{1/2}$ = 20 min rat, s.c), it has the ability to distribute into the brain and spinal cord [213]. It has been observed that the site of action of CP-101,606 is most likely in the brain and not in spinal locations. This was concluded based on anti-hyperalgesia from intracerebroventricular administration (CCI model) and the lack of an anti-hyperalgesia effect when administered intrathecally [214]. Similar to the glycine-site antagonists, reports have emerged which suggest an acceptable therapeutic window for this class of compounds [141].

ALKYL GUANIDINES

Several alkyl guanidine compounds have been reported to have NMDA binding activity and antinociceptive activity (Fig. 8). These compounds are complex to understand, since they tend to have substantial ancillary pharmacology. However a summary of pain related activities in context to NMDA receptor binding is included.

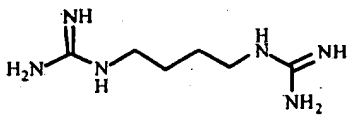
Arcaine sulfate (ARCA) caused hyperalgesia in thermal flexion reflex tests. It was ineffective in formalin and mechanical flexion reflex test but did not cause motor dysfunction at the highest dose studied [151]. Similar to agmatine, it is proposed to bind the open channel [215, 216].

Agmatine is reported to be an endogenous antagonist of NMDA receptors, however it has activity associated with other receptors such as imidazoline and α_2-adrenergic receptors [217]. Agmatine is suggested to be acting *via* an open channel block, and is competitive with MK-801 [216]. Other studies link agmatine to the polyamine site. The K_i for spermine-potentiated [^{3}H] MK-801 binding is 14.8 μM compared to > 500 μM for direct displacement of [^{3}H] MK-

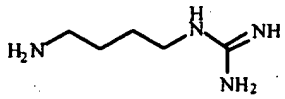
Table 4. Pain Data Associated with Polyamine Site/Ifenprodil Site Antagonists

Antagonist/ *In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
Ifenprodil IC_{50} = 0.250 μM @NR2B; >40 μM @NR2A [81]	Mechanical nociception, carrageenan	i.p.	r	(+)		[64]
	Mechanical allodynia, nerve injury	i.p.	r	(+)	(-)	[141]
	Thermal hypersensitivity, PGE2	i.p.	r	(-)		[212]
(+/-)Ro 25-6981 IC_{50} = 0.009 μM @NR2B; > 60 μM @ NR2A [81]	Mechanical allodynia, nerve injury	i.p.	r	(+)	(+)	[141]
(+/-)-CP-101,606 IC_{50} = 0.060 μM @NR2B; >100 μM @NR2A [81]	Mechanical hyperalgesia, carrageenan	s.c	r	(+)	(+)[c]	[213]
	Capsaicin-induced nociception	s.c	r	(+)	(+)[c]	[213]
	PMA-induced nociception	s.c	r	(+)	(+)[c]	[213]
	Mechanical allodynia, nerve injury	p.o.	r	(+)	(+)	[141]
	Mechanical hyperalgesia, carrageenan	p.o	r	(+)	(+)	[141]

[a] Route of administration and species tested: i.p. = intraperitoneal,; r = rat. [b] Result : (+) = postive result, (-) = negative result. Side effect separation (+) = no noticeable effects in assay, (-) = noticeable effects, no value = not reported.[c] Therapeutic margin observed between *in vivo* pain assay and motor function assay.



ARCA
Arcaine



Agmatine

Fig. (8). Alkyl guanidines.

801 [218]. It possesses activity (i.p.) in early phase and late phase formalin. However, the activity of agmatine in late phase formalin but not early phase can be blocked by yohimbine suggesting a role of α2 receptors. There was no reported activity in tail flick model in this same study [219]. Agmatine is known to be an allosteric modulator of α2A receptors (~10 μM), which can mediate nociception as well [220]. A recent study has suggested that (*i.th.*) antinociceptive effect of agmatine in a tail pinch model is related to the imidazoline receptors [221]. Agmatine has shown efficacy in a carrageenan-evoked mechanical hyperalgesia, dynorphin-induced allodynia and nerve-injury models (Chung model, *i.th.*, admin.) with no effect on behavior changes. There was no effect in acute pain tests. The authors suggest that agmatine may play an endogenous role in regulating neuropathic pain [222].

CONCLUSION

NMDA antagonists are well-studied in a variety of neuropathic pain models, and results suggest they may be useful for treating the pathological conditions underlying neuropathic pain while not affecting the normal physiological pain responses. However, deleterious side-effects observed with many of the compounds have raised the question if this is a mechanism-based effect which cannot be overcome. It appears that within the non-competitive class of NMDA receptor antagonists, the most potent compounds (*e.g.* MK-801) are unsuitable for clinical use due to the side effect profile. Low affinity non-competitive antagonists may be more useful, with fewer observable side effects. For example, memantine has demonstrated a superior side-effect profile, and is currently in use for other diseases. However, memantine did not show efficacy in several models of clinical pain.

Advances in the competitive NMDA class of compounds have led to the discovery of the peptides Con-G and Con-T which represent NR2B subtype selective competitive antagonists with the potential for improved side-effect profiles. However, the peptidic nature of these compounds would certainly diminish the chances of these compounds being orally efficacious treatments for pain. The glycine site-antagonists also represent a potential area for future pain therapies. Many of the initial glycine-site antagonists suffered from poor physicochemical properties that may preclude future clinical development and may have complicated the *in vivo* assays. More recently, orally efficacious glycine-site antagonists have been discovered that demonstrate good efficacy in animal models of neuropathic pain. Ongoing clinical trials with NMDA antagonists in neuropathic pain will help further establish if these types of compounds are useful in clinical practice. Finally, emerging literature on the role of peripheral NMDA receptors in neuropathic pain may lead to a better understanding of how CNS-mediated side effects of NMDA-antagonists might be avoided.

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ABBREVIATIONS

FCA	=	Freund's complete adjuvant.
CCI	=	Chronic constrictive nerve injury
BBB	=	Blood-brain barrier
EP	=	Early phase (formalin assay)
LP	=	Late Phase (formalin assay)

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Excitatory amino acid antagonists and their potential for the treatment of ischaemic brain damage in man

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1 A wide range of therapeutic strategies has been explored in humans and experimental animals with the aim of improving outcome after brain ischaemia but few have shown convincing clinical benefit.

2 The massive increase in the extracellular concentration of glutamate which occurs in cerebral ischaemia is a key component in the sequence of neurochemical events which leads to neuronal death. Pharmacological blockade of the action of glutamate at the *N*-methyl-D-aspartate (NMDA) receptor, (the glutamate receptor subtype principally involved in the neurotoxic effects of the amino acid) provides a novel therapeutic approach to cerebral ischaemia.

3 The effects of NMDA receptor antagonists in animal models of focal cerebral ischaemia are uniquely consistent, *viz*, a marked reduction in the amount of irreversible ischaemic damage irrespective of the species, the model of cerebral ischaemia, when the animals are sacrificed after the ischaemic episode, whether ischaemia is permanent or temporary and followed by reperfusion and which particular NMDA antagonist was employed.

4 NMDA receptor antagonists have marked effects on brain function in normal animals. The balance between these potential adverse effects and the anti-ischaemic efficacy of these drugs will ultimately determine the clinical utility of this class of drugs.

5 The data which are reviewed provide the basis for the current clinical evaluation of NMDA receptor antagonists in stroke and head trauma.

Keywords *N*-methyl-D-aspartate ischaemic brain damage glutamate receptor

Introduction

The importance of cerebral ischaemia is a reflection of the frequency of cerebrovascular disease in advanced societies and the severity of its sequelae. Cerebrovascular disease ranks third (after cancer and heart disease) as the cause of death in Western Europe and North America and is the major cause of handicap in the adult population. Approximately 500,000 people in the U.K. are presently incapacitated by the neurological effects of cerebral ischaemia.

Focal cerebral ischaemic damage (stroke) results from a reduction in cerebral blood flow to a discrete brain area. The origin of the ischaemic episode may be occlusive (due to *in situ* arterial thrombosis), embolic or haemorrhagic. In some patients it is due to a combination of proximal vascular narrowing and impairment of total cerebral blood flow, e.g. due to a sudden reduction in cardiac output. Ischaemic brain damage is a feature of a number of clinical conditions other than stroke, most notably head injury, prolonged seizures, cardiac arrest, perinatal hypoxia, etc. These conditions provide additional patients who may benefit from excitatory amino acid receptor antagonists and, as in the case of head injury, clinical populations in which the efficacy and potential adverse reactions of these class of drugs may be readily studied.

Stroke therapy can be directed at a wide range of pathophysiological mechanisms and there has long been particular interest in medical and surgical therapies designed to improve cerebral blood flow to the ischaemic tissue. Drugs which putatively increase flow to ischaemic tissue, such as nimodipine, are of clear benefit to subarachnoid haemorrhage patients who are at high risk of

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delayed ischaemia due to vasospasm and reduced cerebral blood flow (Pickard *et al.*, 1989). Nimodipine may also be of benefit in stroke, but the evidence is more controversial (Gelmers *et al.*, 1988). In addition, there has long been concern that simply increasing blood flow to ischaemic brain tissue may have adverse consequences such as increased cerebral oedema, haemorrhagic transformation or generation of free radicals (Hossmann, 1982).

The role of excitatory amino acids in the genesis of ischaemic neuronal injury

The concept that blockade of excitatory amino acid receptors attenuates the transmembrane ionic fluxes that lead to neuronal death provides a therapeutic strategy that does not depend upon improvement in cerebral blood flow. High concentrations of glutamate are neurotoxic (Choi, 1991; Lucas & Newhouse, 1957; Rothman & Olney, 1986). From extensive investigations in cell cultures (for review see Choi, 1991), the neurotoxic effects of glutamate appear to be mediated predominantly via activation of the *N*-methyl-D-aspartate (NMDA) receptor subtype although the contribution of non-NMDA receptors is becoming increasingly recognised (see Choi, 1991; Choi *et al.*, 1988; Frandsen *et al.*, 1989; Michaels & Rothman, 1990). Recent evidence suggests that the generation of nitric oxide via NMDA receptor activation may contribute to neuronal damage (Dawson *et al.*, 1991).

In experimental cerebral ischaemia, there is a marked, immediate increase in the extracellular concentrations of glutamate and aspartate, irrespective of the nature and primary cause of the ischaemic episode (Figure 1). Ischaemia induced elevations in excitatory amino acids occur in all brain areas which have been investigated and in response to all experimental approaches employed to provide low levels of cerebral blood flow (i.e. global ischaemia, middle cerebral artery occlusion, CNS trauma, subdural haemorrhage) (McCulloch *et al.*, 1991). The elevation in extracellular glutamate in ischaemia is due to an increased release from neurones, to an impaired uptake of glutamate into neurons and astrocytes in the ischaemic tissue and to reversal of the uptake mechanism (Nicholls & Attwell, 1990). The relationship between extracellular glutamate and cerebral blood flow is a threshold type relationship with elevation in glutamate being triggered by blood flow reduction below 20 ml 100 g^{-1} min^{-1} (Shimada *et al.*, 1989), suggesting that glutamate threatens cerebral tissue in the ischaemic penumbra as well as in the ischaemic core. The blood flow threshold for irreversible damage to neurones is time dependent. Cerebral blood flow of 17 ml of blood 100 g^{-1} of brain tissue min^{-1} (or 35% of basal levels of cerebral blood flow) must be sustained for 3 h or more to produce damage, whereas neuronal damage occurs if there is a complete cessation of cerebral blood flow beyond a few minutes (Jones *et al.*, 1981).

The actions of excitatory amino acids such as aspartate and glutamate are mediated by at least four distinct receptor subtypes. NMDA, kainate and 2-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors have been defined in terms of their selective affinity for the appropriate agonists and these glutamate receptor subtypes are all associated with receptor operated ion channels. A fourth glutamate receptor subtype ('the metabotropic receptor') has been identified recently and is linked to phosphoinositide metabolism (Lodge & Collingridge, 1990).

There are a number of distinct sites within the NMDA receptor ion channel complex at which drugs may act to attenuate the effects of glutamate (Figure 2) (see Foster & Fagg, 1987). Conceptually, the most simple site at which NMDA antagonists can exert their action is the neurotransmitter recognition site for glutamate and NMDA, the most potent of these competitive NMDA

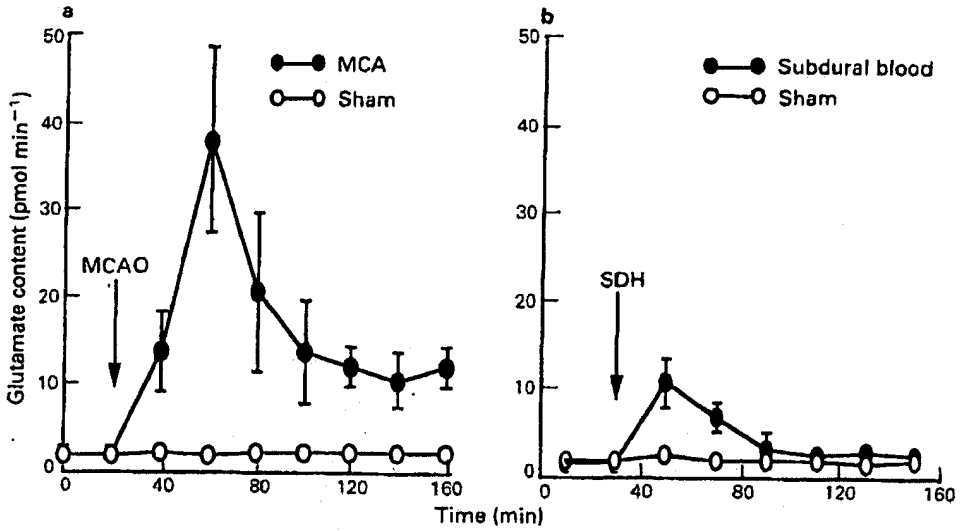

Figure 1 Extracellular glutamate concentrations are elevated in focal cerebral ischaemia produced by middle cerebral artery occlusion (a) and after induced subdural haematoma (b). Data are from microdialysis probes in the rat cerebral cortex. Dialysates were collected in 20 min fractions (2.5 μl min^{-1}). After middle cerebral artery occlusion, there is approximately a 20-fold increase at peak in extracellular glutamate concentrations; after subdural haematoma, there is a five-fold increase. Redrawn from the data of Butcher *et al.* (1990) and Bullock *et al.* (1991).

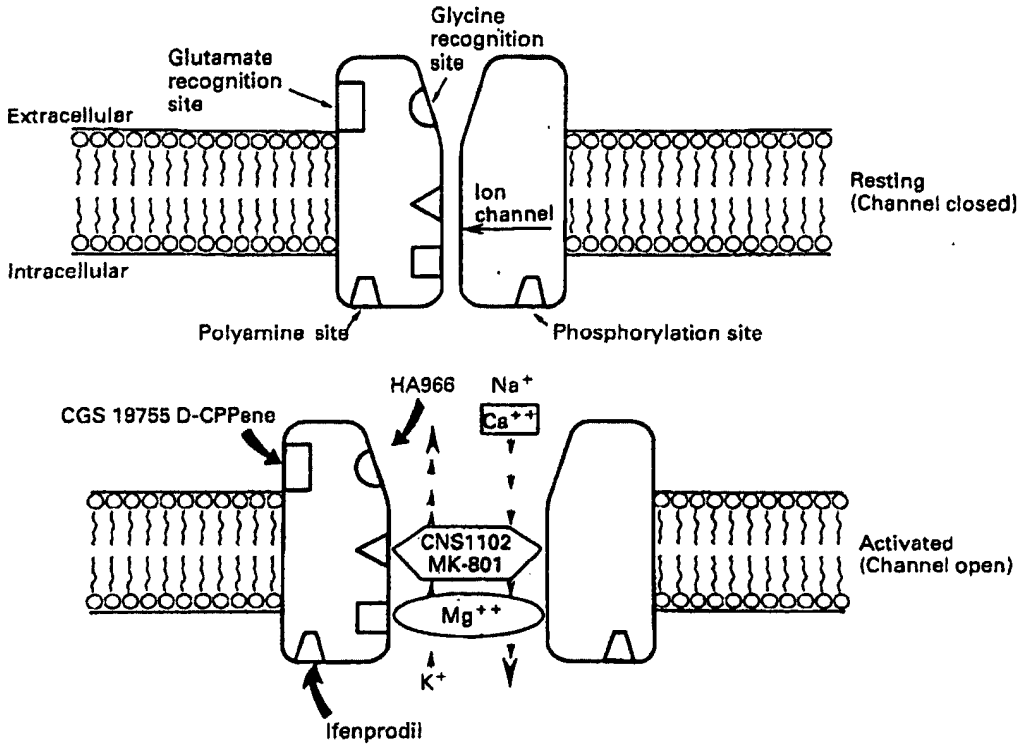

Figure 2 Diagrammatic representation of the NMDA receptor complex. Blockade of the NMDA receptor can be achieved at multiple, pharmacologically distinct sites. Competitive NMDA antagonists (e.g. D-CPPene, CGS 19755) act at the agonist recognition site. Non-competitive NMDA antagonists (e.g. MK-801, CNS 1102) and Mg^{++} act at distinct sites within the ion channel. Blockade of the actions of NMDA can also be achieved via blockade of the glycine recognition site (e.g. with HA-966) or polyamine site (e.g. with ifenprodil). Redrawn from Foster & Fagg (1987).

antagonists which have been studied in models of ischaemia being *cis*-4-phosphonomethyl-2-piperidine-carboxylic acid (CGS 19755) and D-3(2-carboxypiperazin-4-yl) propenyl-1-phosphonic acid (D-CPPene) (Aebischer *et al.*, 1989; Lehmann *et al.*, 1988). Agents such as MK-801, CNS 1102 and phencyclidine (PCP) interact with a site within the ion channel of the NMDA receptor to produce a non-competitive blockade of the actions of glutamate (Kemp *et al.*, 1987). Agents such as 7-chloro-kynurenic acid and 3-amino-1-hydroxy-2-pyrrolidone [(+)HA 966] appear to attenuate the effects of NMDA receptor agonists by acting at a site through which glycine allosterically enhances NMDA receptor function (Kemp *et al.*, 1988; Singh *et al.*, 1990). Other allosteric sites within the NMDA receptor (the 'polyamine site') may be involved in the action of ifenprodil and related compounds to the NMDA receptor complex (Carter *et al.*, 1989). The opening of the NMDA receptor-ion channel is voltage-dependent by virtue of blockade with physiological concentrations of magnesium; membrane depolarisation at the onset of cerebral ischaemia relieves the magnesium block of the NMDA ion channel.

The existence of multiple, pharmacologically active sites within the NMDA receptor ion channel complex is not of esoteric neuropharmacological interest. The different sites within the NMDA receptor complex at which non-competitive antagonists (such as MK-801) and competitive antagonists (such as D-CPPene) act, and the influence of glutamate upon their interactions with their specific binding sites may have a crucial bearing on the efficacy of these two types of NMDA antagonists in cerebral ischaemia and their potential for adverse effects on CNS function. Non-competitive antagonists such as MK-801 produce a use-dependent blockade, in which the binding of the drug to its recognition site in the ion channel and the resulting NMDA blockade are markedly enhanced by high concentrations of glutamate (Kemp *et al.*, 1987; Wong *et al.*, 1986). In contrast, the NMDA receptor blockade produced by competitive antagonists such as DCPPene can be overcome or reduced by increasing concentrations of glutamate (Kemp *et al.*, 1987). In cerebral ischaemia the presence of high extracellular glutamate levels should intensify the blockade produced by non-competitive NMDA antagonists such as MK-801, but could potentially counteract the blockade produced by competitive antagonists such as D-CPPene.

Anti-ischaemic efficacy of NMDA receptor antagonists in experimental animals

Focal cerebral ischaemia

The effects of NMDA receptor antagonists in experimental models of focal cerebral ischaemia can be readily summarised, *viz.* these drugs effect a marked reduction in the amount of irreversible ischaemic damage irrespective of the species, the model of cerebral ischaemia, when the animals are sacrificed after the ischaemic episode, whether ischaemia is permanent or temporary and followed by reperfusion, and irrespective of the particular site within the NMDA receptor at which the drug acts.

The consistency of view which has emerged from the

use of NMDA antagonists in experimental focal ischaemia is unique for any pharmacological class of anti-ischaemic drug. The anti-ischaemic efficacy of NMDA antagonists in experimental focal ischaemia (as distinct from global ischaemia, *vide infra*) is not due to the focality of the ischaemic insult but to its moderate severity as distinct from the complete (or near complete) absence of blood flow to the brain in most reliable global models. In middle cerebral artery occlusion models of focal ischaemia, the failure of NMDA antagonists to protect the basal ganglia has been attributed to the much lower levels of blood flow which occur after occlusion of the middle cerebral artery in the caudate nucleus relative to the cerebral cortex. The lack of protection in the caudate nucleus indicates that a minimal level of cerebral blood flow is required for anti-ischaemic efficacy of NMDA antagonists (McCulloch *et al.*, 1991).

Cat The clearest evidence of the potency of NMDA antagonists as anti-ischaemic agents has emerged from studies of their effects in permanent middle cerebral artery (MCA) occlusion in the cat where the volume of ischaemic damage has been comprehensively assessed (Figure 3). Pretreatment with a competitive antagonist (D-CPPene) or a non-competitive antagonist (MK-801) or polyamine site blockers (ifenprodil and *d*-(4-chlorophenyl)-4-[(4-fluorophenyl)methyl]-1-piperidine ethanol (SL 82.0715)), administered within 5 min of the occlusion, markedly reduces the volume or irreversible ischaemic brain damage in the cerebral hemisphere (Figure 4) (Bullock *et al.*, 1990; Chen *et al.*, 1991; Gotti *et al.*, 1988; Ozyurt *et al.*, Uematsu *et al.*, 1991).

A critical issue for all potential anti-ischaemic compounds is that of how long after the onset of the ischaemic episode these agents are able to prevent ischaemic damage occurring. It is self-evident that for a drug to be effective it must be present in the ischaemic tissue in adequate concentration during the time window of the therapeutic

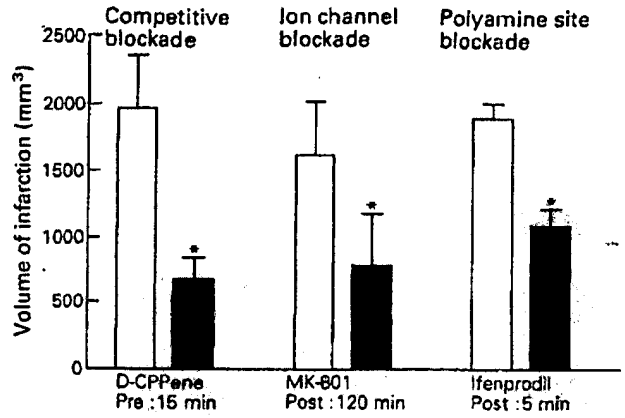

Figure 4 NMDA receptor antagonists markedly reduce the volume of ischaemic brain damage which results from permanent middle cerebral artery (MCA) occlusion. The magnitude of neuroprotection is similar with competitive blockade (D-CPPene, 15 mg kg^{-1}, i.v. 15 min before MCA occlusion), ion channel blockade (MK-801 5 mg kg^{-1}, i.v. 120 min after MCA occlusion), and polyamine site blockade (ifenprodil, 16.7 μg kg^{-1} min^{-1}, i.v. initiated 5 min after MCA occlusion). Data are presented as mean ± s.e. mean (n = 6–13 per group). □ vehicle, ■ drug. Original data are from Chen *et al.* (1991), Gotti *et al.* (1988) and Park *et al.* (1988). Reproduced from McCulloch & Iversen (1991) with permission.

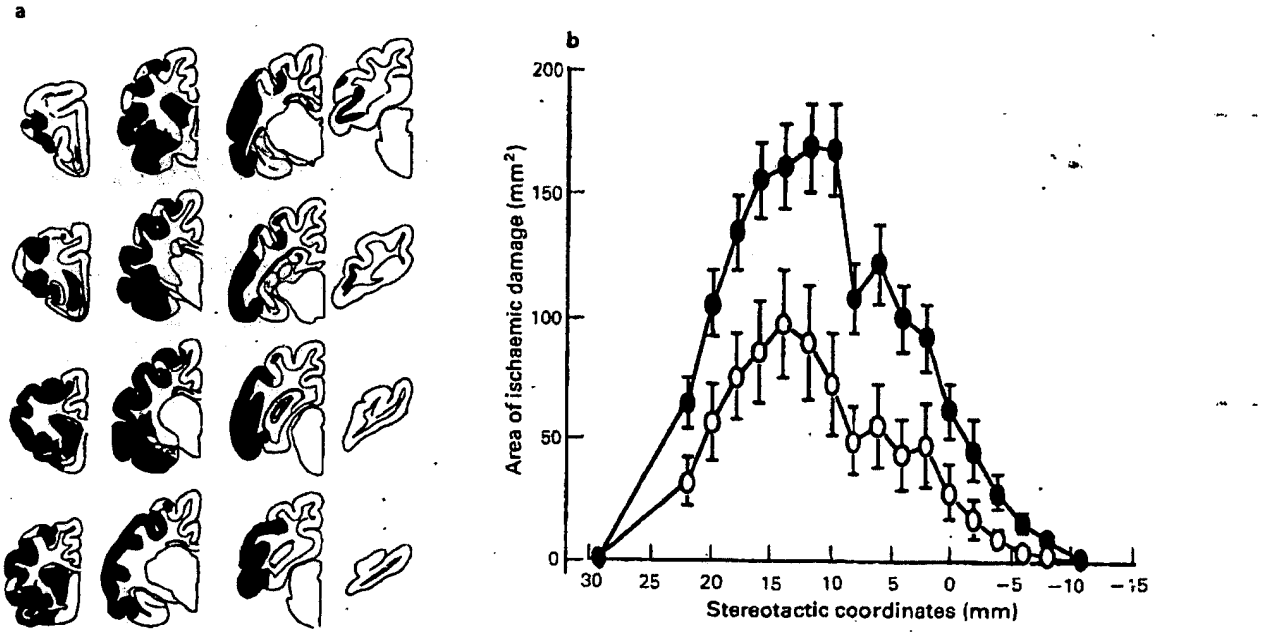

Figure 3 Effect of MK-801 upon ischaemic brain damage after middle cerebral artery occlusion in the cat: volumetric assessment of ischaemic brain damage. a) The areas of ischaemic brain damage (solid black) assessed with light microscopy, are charted onto line drawings for 16 predetermined coronal planes. b) Effect of MK-801 (5 mg kg^{-1} 30 min prior to MCA occlusion) on the area of ischaemic damage in the 16 coronal planes. There are significant differences between vehicle (•) and MK-801 (○) treatment at each coronal plane. Data are mean ± s.e. mean (n = 9 in each group). The volumes of ischaemic damage calculated from the areas and the known stereotactic co-ordinates were vehicle 3231 ± 394 mm^3 and MK-801 1602 ± 445 mm^3 ($P < 0.01$). Original data from Ozyurt *et al.* (1988) reproduced from McCulloch *et al.* (1991) with permission.

opportunity (i.e. less than 3 h in the cat MCA occlusion model (even with penumbra level of blood flow). The chemistry of the drug has considerable bearing on what extent (and how quickly) plasma drug levels are reflected in ischaemic cerebral tissue. For a highly lipophilic agent such as MK-801 with rapid CNS entry, the low levels of blood flow in ischaemic tissue only slightly delay its appearance in ischaemic tissue (e.g. 5 min after administration, the level in ischaemic tissue is 50% of that in the cerebellum) (Wallace *et al.*, 1992). By virtue of its rapid brain uptake, MK-801 first administered 2 h after the onset of ischaemia is as effective as pretreatment in reducing the volume of ischaemic brain damage in the cat MCA occlusion model (Park *et al.*, 1988b). In contrast for hydrophilic molecules such as D-CPPene (and almost all other competitive NMDA antagonists presently available) the rate at which equilibrium is achieved between plasma and CNS is extremely slow (half-time of CNS uptake of 60 min or more). The slow diffusion across the blood-brain barrier probably accounts for the lack of a significant effect of D-CPPene when treatment is initiated 1 h after MCA occlusion (Chen *et al.*, 1991).

There is evidence which suggests that the magnitude of neuroprotection offered by MK-801 is broadly similar in temporary MCA occlusion in the cat (2 h occlusion followed by 4 h reperfusion) (Uematsu *et al.*, 1991) to that observed with 6 h of permanent MCA occlusion (Ozyurt *et al.*, 1988; Park *et al.*, 1988a). Furthermore, nimodipine treatment together with MK-801 appears to result in greater reductions in the amount of brain damage than does MK-801 alone in the cat focal ischaemia – reperfusion model (Uematsu *et al.*, 1991).

Primate In the single study available at present, post-ischaemic treatment with MK-801 reduces the amount of brain damage and improves neurological outcome after temporary focal ischaemia in non-human primates (Zabramski *et al.*, 1991).

Rabbit The investigations of the efficacy of NMDA antagonists in focal cerebral ischaemia in rabbits, though numerically limited, contain a number of interesting features. They provide one of the few reliable demonstrations of the efficacy of MK-801 in a model of embolic stroke (Kochhar *et al.*, 1988). Functional recovery after MK-801 treatment in spinal cord ischaemia was first shown in the rabbit (Kochhar *et al.*, 1988). Dextromethorphan and its active metabolite, dextrorphan, which are weak, non-competitive NMDA antagonists, have been most extensively examined in a rabbit model of temporary focal ischaemia followed by reperfusion. Both these agents, with pretreatment and with treatment initiated at the start of reperfusion after 1 h of ischaemia, reduce the amount of ischaemic damage (assessed with histology), the amount of oedema (assessed with MRI) and improve functional recovery (assessed with somatosensory evoked responses) (George *et al.*, 1988; Steinberg *et al.*, 1988, 1991). It should be emphasised that the threshold anti-ischaemic dose of these agents in the rabbit is 15 mg kg^{-1} (i.v.) in the first hour of treatment (Steinberg *et al.*, 1991), compared with the antitussive dose in man of 0.2–0.4 mg kg^{-1} by mouth (3–4 times daily).

Rat The efficacy of NMDA antagonists in rat models of focal cerebral ischaemia has been confirmed in numerous reports. There is overwhelming evidence that non-competitive NMDA antagonists (MK-801, TCP, PCP) reduce the amount of ischaemic damage after MCA occlusion in the rat (Bielenberg & Beck, 1991; Dirnagl *et al.*, 1990; Gill *et al.*, 1991; Gotti *et al.*, 1988; Park *et al.*, 1988a; Roussel *et al.*, 1992). There is growing evidence for the view that competitive NMDA antagonists, glycine antagonists, polyamine site antagonists and the systemic administration of Mg^{++} are also effective in focal ischaemia in the rat (Gill *et al.*, 1991; Gotti *et al.*, 1988; Izumi *et al.*, 1991; Park *et al.*, 1991; Simon & Shiraishi, 1990). The volume of tissue which can be salvaged from irreversible ischaemic damage with NMDA antagonists is approximately 50% of the infarction volume in untreated rats; the maximum anti-ischaemic effects of the drugs are broadly similar irrespective of their precise site of action within the NMDA receptor complex. Marked neuroprotection with MK-801 and PCP is observed despite the marked hypotension which it produces in halothane-anaesthetised rats (Bielenberg & Beck, 1991; Park *et al.*, 1988a). Hypotension would tend to exacerbate damage by reducing blood flow in the ischaemic penumbra to even lower levels (Osborne *et al.*, 1987). Drug-induced hypotension is the probable cause of the U-shaped dose-response curve noted with PCP and MK-801 in the rat MCA occlusion model (Bielenberg & Beck, 1991; Gill *et al.*, 1991). There is evidence (see Dirnagl *et al.*, 1990; Roussel *et al.*, 1990, 1992) that the magnitude of response to MK-801 and kynurenate may be somewhat smaller in spontaneously hypertensive animals probably because the ischaemic insult after MCA occlusion is more severe in the hypertensive strain than in normotensive animals (Roussel *et al.*, 1992).

A recent report indicates that blockade of glutamate receptors other than the NMDA subytpe with NBQX can also reduce ischaemic damage in the rat (Gill *et al.*, 1992).

Perinatal hypoxia

Perinatal hypoxia, like focal cerebral ischaemia, is another area where there is convincing evidence of reductions in ischaemic brain damage. MK-801, kynurenate and dextromethorphan all putatively reduce the amount of damage produced by hypoxia and unilateral carotid artery occlusion in neonatal rats (Andiné *et al.*, 1988; Hattori *et al.*, 1989; McDonald *et al.*, 1987; Olney *et al.*, 1989; Prince & Feesser, 1988). MK-801 treatment is of benefit even when initiated up to 75 min after the hypoxic episode (Hattori *et al.*, 1989; McDonald *et al.*, 1989). Despite their undoubted efficacy in neonatal models of hypoxia, the medico-legal problems associated with administration of new drugs to brain damaged infants effectively preclude the use of NMDA antagonists in this clinical area at present (particularly within litigatious North America).

Global cerebral ischaemia

The pivotal investigations on anti-ischaemic efficacy of selective NMDA antagonists were that MK-801 could

protect the hippocampus of the gerbil from the effects of global ischaemia (Gill *et al.*, 1987). The present status of NMDA receptor antagonists in animal models of global ischaemia can be summarised readily. In all of the studies of global ischaemia in large animals (dogs, cats and primates) no benefit has been demonstrated. In studies of global ischaemia in rodents (rats and gerbils), while the balance of evidence, in numerical terms, favours the view that NMDA antagonists reduce delayed damage to the hippocampus, all positive reports attract criticisms that the benefit observed is indirect (i.e. due to anticonvulsant effects, drug-induced hypothermia). The severity of the ischaemia between different models of global ischaemia appears to provide the best explanation for divergent observations between different investigations (McCulloch *et al.*, 1991).

Only MK-801 has been systematically studied in large animal models of global ischaemia. In dogs, MK-801 fails to alter neurological deficits and the amount of hippocampal damage produced by 11 min global ischaemia (occlusion of ascending aorta) (Michenfelder *et al.*, 1989) or a model of prolonged (17 min) cardiac arrest with a variety of treatment paradigms (Sterz *et al.*, 1989). Similarly, in cats, MK-801 does not improve outcome (neurological deficit and neuropathology in the cortex, hippocampus and cerebellum) after 18 min cardiac arrest (Fleischer *et al.*, 1989). In a study of non-human primates with 17 min of ischaemia, MK-801 again did not provide any evidence of amelioration of the ischaemic damage to the CNS (Lanier *et al.*, 1990).

The influence of excitatory amino acid antagonists on the delayed degeneration of hippocampal CA1 pyramidal neurons in the gerbil and the rat has been the subject of intense investigation and controversy. A clear understanding of the biological and technical bases for divergent results from different laboratories is beginning to emerge. The crucial determinant of whether NMDA antagonists will be effective in ischaemia (whether focal or global) appears to be the severity of the insult and its impact on energy state (see Siesjö & Bengtsson, 1989; Wieloch *et al.*, 1989). In many models of global ischaemia and in the ischaemic core of a focal insult, complete energy failure occurs and NMDA antagonists are not efficacious. In contrast, in the ischaemic penumbra (and possibly in global models where benefit is reported with NMDA antagonists), energy state is less markedly disturbed and NMDA antagonists are clearly efficacious (see Siesjö & Bengtsson, 1989; Wieloch *et al.*, 1989). The difference between partial and complete breakdown of energy production in a diffuse insult like global ischaemia is likely to be highly marginal and extremely sensitive to a number of subtle factors such as anaesthetics, nutritional state, gender, strain (for discussion see Meldrum, 1990). Furthermore, it is now generally accepted that the small differences in brain temperature during and after transient ischaemia dramatically modify the amount of delayed neuronal damage (see Busto *et al.*, 1987; Minanisawa *et al.*, 1990). Buchan & Pulsinelli (1990) produced overwhelming evidence that the ability of MK-801 to provide neuroprotection in the gerbil was inextricably linked to hypothermia. Irrespective of how meticulously temperature is controlled (see Gill & Woodruff, 1990) there will always be concern that drug efficacy is due to hypothermia during the chronic survival period in global ischaemia models (Buchan & Pulsinelli, 1990).

Although NMDA antagonists are not effective in preventing delayed neuronal death after *severe* global ischaemia, NBQX which blocks non-NMDA glutamate receptors has recently been shown to markedly reduce damage to the hippocampus and other brain areas in these severe models (Buchan *et al.*, 1991; Nellgård & Wieloch, 1992).

NMDA receptor antagonists as clinically useful drugs

Excitatory amino acid antagonists are among a wide range of compounds presently being developed as neuroprotective agents. There is an enormous list of drugs and lead compounds at various stages of preclinical or clinical development, e.g. aminosteroids, free radical scavengers, various ion channel blockers, kappa opiate agonists, naftidrofuryl, gangliosides, 5-hydroxytryptamine$_{1A}$ ($5HT_{1A}$) agonists, $5HT_2$-receptor antagonists, α_2-adrenoceptor antagonists, cyclo-oxygenase and lipo-oxygenase inhibitors and others (Ginsberg & Scheinberg, 1991).

It is worth emphasising that among the different pharmacological classes of anti-ischaemic drugs, NMDA antagonists occupy a unique position; for no other class is there such a vast, consistent literature which documents anti-ischaemic efficacy. It is now generally accepted that NMDA blockade reduces brain damage in experimental focal ischaemia irrespective of,

(a) the species used,
(b) the experimental design (anaesthesia, chronic or acute survival, etc.)
(c) the particular site within the NMDA receptor complex at which blockade is achieved,
(d) or whether drug treatment is initiated before or in the first few hours after onset of ischaemia.

Anti-ischaemic efficacy is only one element in the selection of drugs for clinical evaluation. Safety and adverse effects are also of paramount importance in determining the utility of new drugs. It is already clear that the actions of NMDA antagonists (other than those relating to their ability to reduce brain damage) will influence the selection of the clinical target and the design of the clinical trials.

Competitive and non-competitive NMDA antagonists (MK-801 and CPP) depress respiration and induce hypercapnia (Kurumaji *et al.*, 1989). MK-801 increases blood pressure in conscious rats and chloralose-anaesthetised cats (Kurumaji *et al.*, 1989; Ozyurt *et al.*, 1988) but markedly decreases blood pressure in halothane anaesthetised rats (Bielenberg & Beck, 1991; Park *et al.*, 1988a); at high doses, D-CCPene induces hypotension in chloralose-anaesthetised cats (Bullock *et al.*, 1990b; Chen *et al.*, 1991). While these effects present minimal difficulties in some conditions (e.g. head injury patients already on a ventilator in an intensive care unit), in others (e.g. elderly stroke patients with other cardiovascular complications), they may restrict their use.

The administration of NMDA receptors antagonists alters the behaviour of all experimental animals studied hitherto including non-human primates (France *et al.*, 1989; Koek *et al.*, 1988). In primates, the behavioural

effects of NMDA antagonists include disruption of learning and memory, ataxia, sedation and ultimately anaesthesia. The central issue for clinical trials is not whether the drugs induce behavioural changes but the concentration at which the behavioural changes are manifest relative to the therapeutic doses. With non-competitive antagonists typified by MK-801, behavioural alterations are apparent at concentrations similar to those required for anti-ischaemic efficacy. For competitive antagonists, behavioural alterations occur at concentrations three to ten times greater than those required for anti-ischaemic effects and there may be a wider separation for polyamine site antagonists such as ifenprodil (compare the data for the mouse of Koek & Colpaert (1990) and Gotti *et al.* (1990)).

Autoradiographic mapping of the functional consequences of NMDA receptor blockade supports and extends the view which has emerged from behavioural studies. Non-competitive NMDA anatagonists and competitive NMDA antagonists, at doses broadly comparable in terms of anticonvulsant potency and anti-ischaemic efficacy, induce markedly dissimilar alterations in function-related glucose use in the CNS (Kurumaji *et al.*, 1989; Nehls *et al.*, 1988). Pronounced dose-related increases in glucose use were observed throughout the limbic system after non-competitive NMDA receptor antagonists with marked reduction in function-related glucose use widespread in neocortex. In contrast, the effects on glucose use of competitive NMDA receptor blockade or blockade of the glycine site are numerically small and anatomically circumscribed (Hargreaves *et al.*, 1991; Kurumaji *et al.*, 1989).

These alterations in function-related energy generation are particularly important as they appear to be predictive of the reversible morphological alterations which are observed in some brain areas after NMDA antagonists. In the rat posterior cingulate cortex, the acute administration of non-competitive NMDA antagonists, MK-801 phencyclidine and ketamine, effects a dose-dependent cellular swelling and vacuolisation, particularly in the multipolar and pyramidal medium to large sized neurones in layers III and IV. The cellular swelling and vacuolisation subsided 12 h after drug administration, and by 24 h after dizocilpine administration, the histological appearance of the tissue was essentially normal (Olney *et al.*, 1989). These reversible changes in neuronal structure are noted with MK-801 at doses (ED_{50} approx. 0.2 mg kg^{-1}) similar to those at which anti-ischaemic effects, anticonvulsant effects and increased glucose use are seen. Similar neuronal swelling and vacuolisation are also observed with competitive NMDA antagonists when administered intracerebrally and after systemic administration, although doses that are greater than those required to reduce ischaemic damage are necessary (McCulloch & Iversen, 1991).

There are a number of features that should be emphasised in relation to CNS structural changes seen after NMDA receptor blockade. First, these changes are highly circumscribed in their anatomical distribution. Secondly, neither the neuronal swelling nor the increase in glucose use are seen in the posterior cingulate cortex after repeated dizocilpine treatment. Thirdly, the metabolic activation of components of the limbic system after MK-801 and the neuronal swelling and vacuolisation response can be completely prevented by light halothane anaesthesia or centrally acting anticholinergic drugs. Fourthly, the alterations in neuronal structure are completely and rapidly (24 h) reversible (McCulloch & Iversen, 1991). Finally, the risk/benefit ratio in the clinical conditions (stroke, head trauma) in which NMDA antagonists could be used need to be considered; the occurrence of neuronal swelling in a few areas of limbic forebrain, has to be balanced against the normal outcome in stroke and head trauma – at best, significant volumes of cerebral tissue are irreversibly damaged leading to lasting disability or, at worst, the death of the patient. The use of these agents in patients at risk of brain damage is underpinned by the absence of doubt from preclinical investigations that NMDA receptor antagonists will prevent damage occurring to brain tissue in such clinical conditions if administered within a therapeutically relevant time window.

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